BCH 430: BIOCHEMISTRY OF LIPIDS

Lipids:

Lipids are heterogenous group of water –insoluble (hydrophobic) organic molecules that can be extracted from tissues by non-polar solvents such as ether, chloroform, benzene etc and constitute dietary and tissue components.

Lipids constitute a broad group of naturally occurring molecules which include fats, waxes, fat-soluble vitamins (such as vitamins A, D, E and K), monoglycerides, diglycerides, phospholipids, and others. They also encompass molecules such as fatty acids and their derivatives as well as other sterol-containing metabolites such as cholesterol. Although humans and other mammals use various biosynthetic pathways to both break down and synthesize lipids, some essential lipids cannot be made this way and must be obtained from the diet.

Functions of lipids:

Lipids perform several important functions:

- They are the concentrated fuel reserve of the body in the form of triacylglycerols.
- Lipids are the constituents of membrane structure and regulate the membrane permeability (phospholipids and cholesterol).
- They serve as a source of fat-soluble vitamins (A, D, E, and K).
- Lipids are important as cellular metabolic regulators (steroid hormones and prostaglandins).
- They protect the internal organs; serve as insulating materials and give shape and smooth appearance to the body.
- Phospholipids such as phosphatidylserine chelates calcium to act as the foundation for bone growth.
- Phosphatidylcholine acts as a platelet-activating factor, affecting the aggregation of platelets at concentrations as low as 10^{-11}M.
- Bees use wax to produce the rigid structures of their honeycombs.
- Waxes form a thin layer over all green tissue of plants that is both a chemical and a physical barrier. This waxy layer limits the diffusion of water and solutes, while permitting a controlled release of volatiles that may deter pests or attract pollinating insects.

- Fatty acids are the biosynthetic precursors of many insect pheromones and of secondary metabolites in plants.
- Triacylglycerol present in subcutaneous depots serve as insulation against cold in many terrestrial animals, as is obvious in the pig which is surrounded by a layer of fats, and it is also true for marine mammals.
- Again, in marine mammals and fish, the lipid depots are less dense than water and so aid buoyancy with the result that less energy is expended in swimming.
- A specialized form of adipose tissue – the brown fat is highly vascularized, and rich in mitochondria, which oxidize fats so rapidly, generating heat. This heat is very important in young animals and in those recovering from hibernation.
- Lysophosphatidic acid produced by a variety of mammalian cells is highly elavated in patients with ovarian cancer and such; it serves as a useful marker for the early detection of ovarian cancer. Several other functions have been attributed to lipids, making them a very essential biomolecule.

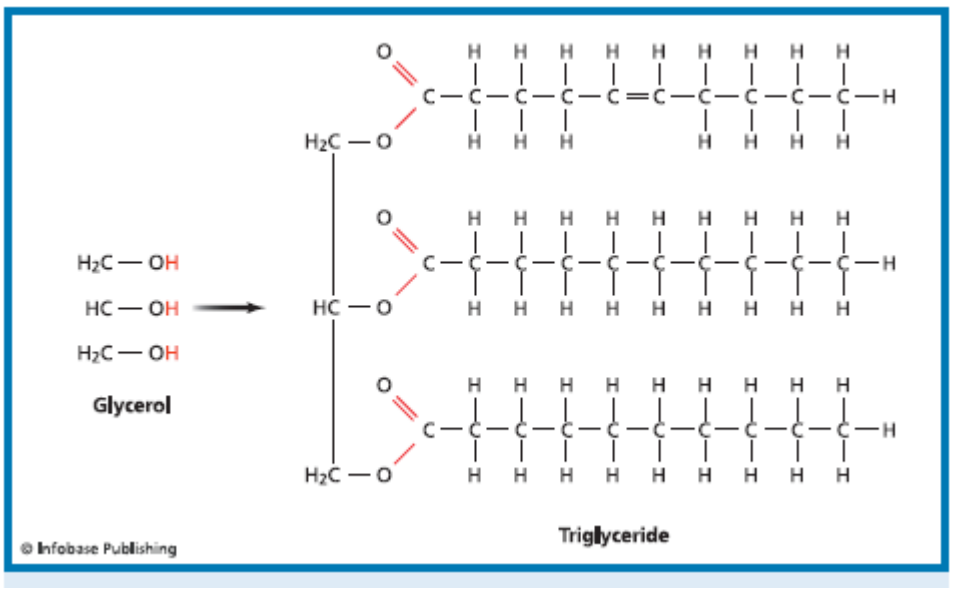
Classification of lipids

Lipids are broadly classified into simple, complex, derived, and miscellaneous lipids, which are further subdivided into different groups.

1. **Simple lipids**

These are esters of fatty acids with alcohols. They are of two types;

Fats and oil or the acylglycerol: Acylglycerol/rides are esters of fatty acids with glycerol.



They are the chemical form in which most fat exists in food as well as in the body. A triglyceride is made up of a three- carbon molecule called glycerol, which is bonded to three fatty acids. Liquid triglycerides are called oils and are found chiefly in plants, although triglycerides from fish are also mostly oils. Triglycerides that are solid or semisolid at room temperature are called fats, and they are mostly found in animals. There are two kinds of fats: saturated and unsaturated.

They have differing properties depending on whether their fatty acids are saturated or unsaturated. For example, saturated fatty acids are typical of animal fats. This is because hibernating and migrant animals store lots of triglycerides for energy reserves. Fats have a lot of energy stored up in their molecular bonds. It is the best way to store energy for long-term use because it provides 9 calories per gram instead of only 4 calories per gram as sugar does. When the body needs extra energy, it breaks down the stored fat. When energy from food is not needed by the body, then it is stored as fat, and when too much fat gets stored, the person becomes overweight.

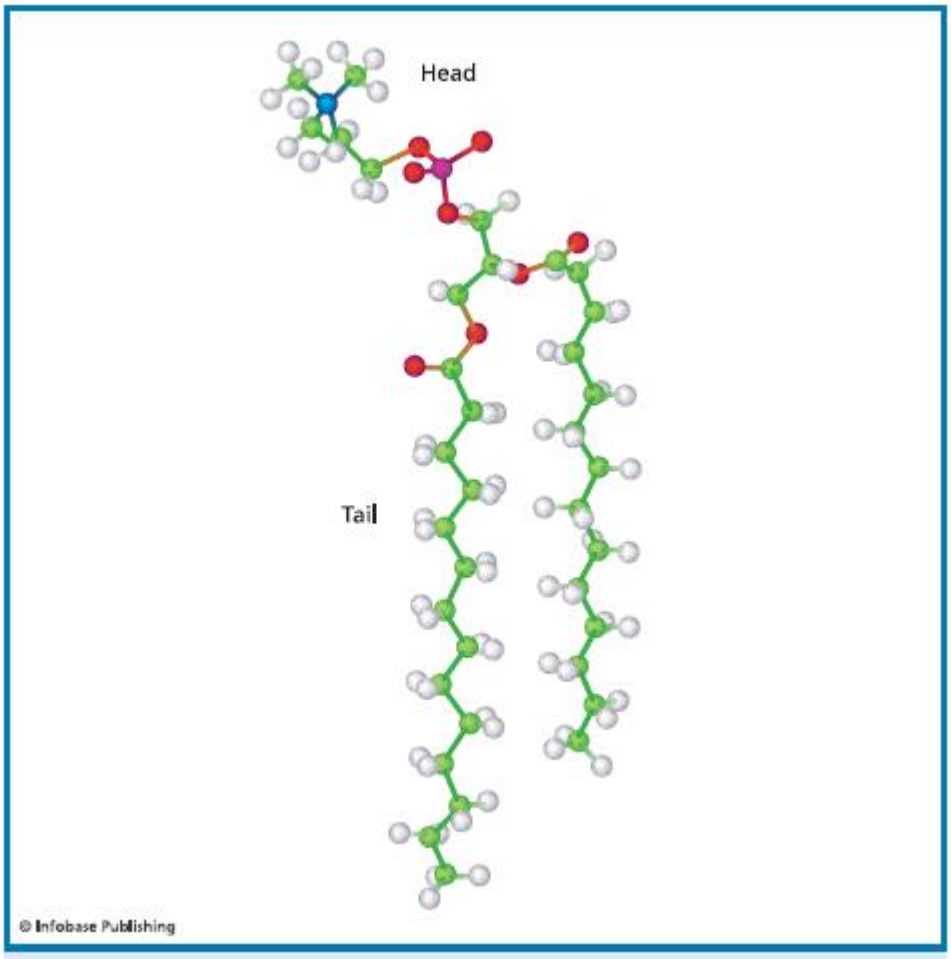
Waxes: These are esters of long chain fatty acids with alcohol other than glycerol. In nature, they serve mostly as protective and structural coatings. Bees make wax to build the walls of honeycombs. Some plants have wax on the outside of their leaves, giving them a shiny appearance. The wax helps cut down on the evaporation of water. The feathers of birds and the fur of some animals have waxy coatings that serve as a water repellent. Humans use wax for the same reason: They wax their skins, their cars, and the floors of their houses to protect them from water and dirt.

2. **Complex or compound lipids**

These are esters of fatty acids with alcohols containing additional groups such as phosphate, nitrogenous base, carbohydrate, protein etc. They are further divided as follows:

a. **Phospholipids:**

These in additions to fatty acid and alcohol contain phosphoric acid and often a nitrogenous base. They are like triglycerides, but they have two fatty acid chains called “tails” and one charged group called the “head” that contains phosphate and oxygen atoms. Because it is charged, the head is Polar and therefore attracts water molecules. The long fatty acid tail is nonpolar and does not attract water molecules. The polar and nonpolar parts of phospholipids allow them to form **lipid bilayers**. “Bi” is from Latin and means “two.” The bilayer forms when the phospholipid molecules arrange themselves in two layers with the tails facing in (facing each other) and the heads facing out.



The result is a phospholipid bilayer that has the tails buried inside and the polar atoms of the heads facing out, where they can form H bonds with water and other molecules.
The membranes of cells are composed of phospholipid bilayers through which small molecules like water and oxygen can pass. Examples of phospholipids include:
Glycerophospholipids (phosphoglycerides): These are phospholipids containing glycerol as the alcohol, e.g lecithin (Phosphatidylcholine), cephalin (phosphatidyl ethanolamine), phosphatidic acid etc. They are the major lipids that occur in biological membranes. They consist of glycerol 3-phosphate esterified at its C_1 and C_2 with fatty acids. Usually, C_1 contains a saturated fatty acid while C_2 contains an unsaturated fatty acid. Phosphatidic acid is the simplest phospholipid which does not occur in good concentration in the tissues. It is an intermediate in the synthesis of triacylglycerols and phospholipids.

Sphingophospholipids: In this group of phospholipids, the alcohol is sphingosine. Example; sphingomyelin. Sphingomyelins are important constituents of myelin and are found in good quantity in the brain and nervous tissues.

b. **Glycolipids (glycosphingolipids):** These lipids contain a fatty acid, carbohydrate and a nitrogenous base. The alcohol is sphingosine; hence they are called glycosphingolipids. Glycerol and phosphate are absent. Examples include; cerebrosides and gangliosides. They are important constituents of the cell membrane and nervous tissues, particularly the brain. Galactocerebroside and glucocerebroside are the most important glycolipids.

3. **Lipoproteins:** Lipoproteins are molecular complexes of lipids with proteins. They are the transport vehicles for lipids in circulation. There are five types of lipoproteins, namely chylomicrons, very low-density lipoprotein (VLDL), low density lipoproteins (LDL), high density lipoproteins (HDL) and free fatty acid- albumin complexes.

4. **Derived lipids**

   These are substances derived from the other two classes of lipids by hydrolysis. They include; fatty acids, glycerol, steroids, fat –soluble vitamins, ketone bodies etc.

   Steroids are compounds containing a cyclic steroid nucleus or ring. It consists of a phenanthrene nucleus (rings A, B, and C) to which a cyclopentane ring (D) is attached.

5. **Miscellaneous lipids**

These include a large number of compounds possessing the characteristics of lipids. Examples are; carotenoids, squalene, terpenes, etc.

Properties and reactions of lipids

Lipids undergo the following chemical reactions:

a) **Hydrolysis:** Enzymatic hydrolysis of triacyglycerols by lipases gives fatty acids and glycerol. Pancreatic lipase breaks the bonds in positions 1 and 3 preferentially to position 2. The phospholipids are hydrolysed by phospholipases. The process of hydrolysis by lipases is important for digestion of fats in the gastrointestinal tract and fat mobilization from the adipose tissues. Hydrolysis of fats by acids gives a free fatty acid and glycerol. Other hydrolysis can be by heat or addition of water.

b) **Saponification:** Hydrolysis of a fat by alkali is called saponification. The products of saponification being glycerol and the alkali salts of the fatty acid called soaps. Triacylglycerol + 3NaOH ====> Glycerol + 3R-COONa (soaps).

c) **Formation of membranes, micelles and emulsions:** Generally, lipids are insoluble in water because of the hydrophobic hydrocarbon chain. The polar lipids (fatty acids and phospholipids) contain a high proportion of polar groups, and are therefore partly soluble in polar solvents, and partly soluble in non –polar solvents. Thus in an oil-water interface,

the molecules become oriented with the polar group in the water phase and the non-polar group in the oil phase forming a bilayer structure.

When a critical concentration of polar lipids is present in an aqueous medium, a micelle is formed.

Emulsions on the other hand are much larger particles formed usually by non- polar lipids in an aqueous medium.

d) **Hydrogenation:** In the presence of a catalyst such as nickel, unsaturated fats are hydrogenated. This process is known as hardening, and is commercially valuable as a method of converting fats mainly of plant origin into solid fats as margarine.

e) **Rancidity:** Rancidity is a term used to represent the deterioration of fats and oils resulting in an unpleasant taste. It occurs when fats and oil are exposed to air, moisture, light, bacteria etc. Hydrolytic rancidity occurs due to partial hydrolysis of triacylglycerol's by bacterial enzymes whereas oxidative rancidity is due to the oxidation of unsaturated fatty acids. The oxygen of the air is believed to attack the double bond of unsaturated fatty acids to form a peroxide linkage. The formation of this linkage results to unpleasant odours and tastes in a fat. Saturated fats are resistant to oxidation and do not exhibit oxidative rancidity. Rancid fats and oils are unsuitable for human consumption.

Antioxidants: These are substances which can prevent oxidative rancidity. Trace amounts of antioxidants such as tocopherols (vitamin E), hydroquinone, gallic acid and α- naphthol are added to commercial preparations of fats and oils to prevent rancidity. Butylated hydroxy-anisole (BHA), propyl gallate, butylated hydroxytoluene (BHT) are the antioxidants used in food preservation.

(f) **Spontaneous oxidation:** Oils which contain highly unsaturated fatty acids example lin-seed oil is spontaneously oxidized by atmospheric oxygen at ordinary temperature, forming a hard water-resistant material. Such oils are used in paints and are referred to as drying oils.

Digestion of Lipids

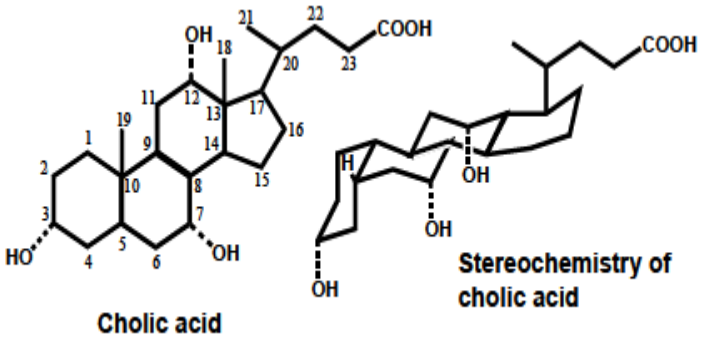
The digestion of lipids is initiated in the stomach by an acid-stable lipase known as lingual lipase which originates from the glands at the back of the tongue. The lingual lipase from the mouth enters the stomach along with the food. It has an optimum pH of 2.5-5; hence it continues to be active in the stomach. It acts on short-chain triglycerides (SCTs) which are mostly present in milk, butter, etc; thus, this enzyme is more active in the newborn infants.

Another enzyme gastric lipase with an optimum pH of about 5.4 is secreted by the stomach mucosa and also acts on short-chain triglycerides. The digestion of lipids in the stomach of an adult is negligible since lipids are not emulsified and made ready for lipase action. Furthermore, the low pH in the stomach is unfavourable for the action of gastric lipase. In infants however, the milk fat with short chain fatty acids can be hydrolyzed by the gastric lipase to some extent. This is because the stomach pH of infants is close to neutrality, ideal for the action of gastric lipase. Up to 30% digestion of triglycerides occurs in the stomach.

Emulsification of Fats- dispersion of dietary fat into small particles with sufficiently exposed surface area for rapid attack by digestive enzymes.

Emulsification is the phenomenon of dispersion of lipids into smaller droplets due to a reduction in the surface tension. This is accompanied by an increase in the surface area of the lipid droplets. Emulsification is a pre–requisite for the digestion of lipids since the enzymes can act only on the surface of lipid droplets (i.e. lipases act at the interfacial area between the aqueous and lipid phase).

Digestion occurs following the dispersion of dietary fat into small particles with sufficiently exposed surface area for rapid attack by digestive enzymes. This is achieved by detergent action and mechanical mixing, with the detergent effect being supplied by several components, both in the diet and in the digestive juices, but especially by partially digested fats (fatty acid soaps and monoacylglycerols) and by bile salts. The free fatty acids, monoacylglycerol, along with phospholipids are known as surfactants. They are characterized by possessing polar and non-polar groups. Surfactants get absorbed into the water-lipid interfaces and increase the interfacial area of the lipid droplets. Thus, the initial action of the lipase enzyme helps in the further digestion of lipids.



Cholic acid

Stereochemistry of cholic acid

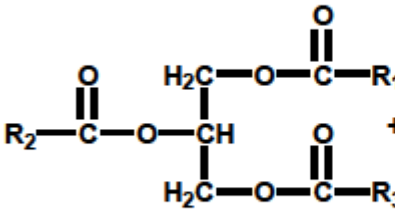
The bile salts such as cholic acid contain a hydrophobic side and a hydrophilic side, thus allowing bile salts to dissolve at an oil-water interface, with the hydrophobic surface in contact with the non-polar phase and the hydrophilic surface in the aqueous medium. This detergent action emulsifies fats and yields mixed micelles, which allow attack by water-soluble digestive enzymes and facilitate the absorption of lipids through the intestinal mucosa. Mixed micelles also serve as transport vehicles for those lipids that are less water-soluble than fatty acids, such as cholesterol or the fat-soluble vitamins A, D, E, and K. Thus, efficient absorption of lipids depends on the presence of sufficient bile acids to solubilize the ingested lipids. Bile salts also stabilize the smaller particles by preventing them from coalescing.

Besides the action of bile acids and surfactants, the mechanical mixing due to peristalsis also helps in the emulsification of lipids.

The presence of free fatty acids in the duodenum causes the release of the peptide hormone **pancreazymin-cholecystokinin** which causes the **gallbladder** to release **bile salts** and the pancreas to release **pancreatic lipase**.

A second protein known as **co-lipase** also secreted by the pancreas in an inactive form called **pro-colipase**, binds the lipase at a ratio of 1:1 and anchores it at the lipid-aqueous interface. Colipase restores activity to lipases in the presence of inhibitory substances such as bile acids. Procolipase is activated by trypsin.

The emulsification of fats renders them susceptible to hydrolysis by enzymes secreted by the pancreas. The most important enzyme involved is **pancreatic lipase**. The pancreatic lipases are primarily responsible for the degradation of dietary triacylglycerol, cholesteryl esters and phospholipids. Pancreatic lipase is virtually specific for the hydrolysis of primary ester linkages, the 1 or the 3 ester bonds of long chain fatty acids of triacylglycerols, but not the bond in the central 2 position (see below). As a result of this conversion, 2-monoglycerides (2-monoacylglycerols) are major end-products of triglyceride digestion. Less than 10% of triglycerides remain unhydrolyzed in the intestine.



Triacylglycerol

Lipid esterase is a less specific enzyme present in the pancreatic juice. It acts on monoacylglycerol, cholesteryl esters, vitamin esters etc to liberate free fatty acids. The presence of bile acids is essential for the activity of lipid esterase. Cholesteryl ester esterase also known as cholesteryl ester hydrolase cleaves cholesteryl esters to produce cholesterol and free fatty acids.

Phospholipases are enzymes responsible for the hydrolysis of phospholipids. Pancreatic juice is rich in phospholipase A_2 which cleaves the fatty acid in the 2^{nd} position of phospholipids. The products are free fatty acids and a lysophospholipid (2-monoacylglycerol-3-P). Phospholipase A_2 is secreted as a zymogen (inactive form), which is activated in the intestine by the action of trypsin.

Absorption of Lipids

Short-chain fatty acids (up to 12 carbons) are absorbed directly through the villi of the intestinal mucosa. They enter the blood via capillaries that eventually empty into the portal vein and are transported via lipid carrier proteins directly to the liver cells, where they are used for energy production. 2-Monoglycerides, long-chain fatty acids (more than 12 carbons), cholesterol and lysophospholipids are absorbed from the lumen by intestinal mucosal cells, where they are incorporated into lipoproteins (chylomicrons) and directed to the lymphatic system. The presence of chylomicrons gives the lymph a milky appearance which is observed after a lipid-rich meal. Within the intestinal wall, the triglycerides are resynthesized by the 2-monoacylglycerol pathway as shown below. The 2-monoacylglycerol pathway is unique for the intestine.

Triglycerides, having been synthesized in the intestinal mucosa, are not transported to any extent in the portal venous blood. Instead, the great majority of absorbed lipids, including triglycerides, phospholipids, cholesterol esters, and cholesterol, appear in the form of chylomycrons that pass to the lymphatic vessels of the abdominal region and later to the systemic blood, then to the peripheral tissues (muscle and adipose tissue), and finally to the liver. Adipose tissues and muscles take up a large proportion of dietary lipids from chylomicrons for storage and transport. This bypass arrangement of

chylomicrons through the peripheral tissues protects the liver from lipid overload after a meal.

KEY POINTS: DIGESTION AND ABSORPTION OF LIPIDS

- Bile salts help in the digestion of fats, cholesterol and fat-soluble vitamins by forming mixed micelles which solubilize the fats and render them accessible to digestive enzymes.
- The presence of free fatty acids in the duodenum causes release of the peptide hormone pancreazymin-cholecystokinin which causes the gallbladder to release bile salts and the pancreas to release pancreatic lipase.
- Pancreatic lipase hydrolyzes triglycerides (triacylglycerols) to give free fatty acids and *2-monoglycerides* which are absorbed by the intestinal mucosa cells.
- Short-chain fatty acids ($\geq$12) enter the portal vein and are transported directly to the liver.
- All other fatty acids are re-esterified with the 2-monoglycerides to form triglycerides which are incorporated into lipoprotein particles called chylomicrons which enter the lymphatic vessels.

Abnormalities of lipid digestion and absorption

Steatorrhea: This is a condition characterized by loss of lipids in the faeces. Steatorrhea may be due to a defect in the secretion of bile or pancreatic juice into the intestine; or due to impairment in the lipid absorption by the intestinal cells. Steatorrhea is commonly seen in disorders associated with pancreas, biliary obstruction, severe liver dysfunction, etc.

Cholesterol stones: Cholesterol stone formation in gall bladder (gall stones) is a frequent health complication found in females than in males often in association with obesity. Cholesterol gall stones are formed when the liver secrets bile containing phospholipids, bile acids etc, and supersaturated with cholesterol.

Lipoproteins

A **lipoprotein** is a biochemical assembly that contains both proteins and lipids. They function as transport vehicles for lipids in blood plasma. Lipoproteins deliver the lipid components (cholesterol, triacylglycerol, phospholioids) to various tissues for utilization. Lipoproteins basically consist of a neutral lipid core, surrounded by a coat of phospholipids, apoproteins and cholesterol. The polar portions of phospholipids and cholesterol are exposed on the surface of lipoproteins such that lipoproteins are soluble in aqueous solution. Many enzymes, transporters, structural proteins, antigens, adhesins and toxins are lipoproteins. Examples include the high density (HDL) and low density (LDL) lipoproteins which enable fats to be carried in the blood stream.

Classification

By density

Lipoproteins may be classified as follows, listed from larger and less dense to smaller and denser. Lipoproteins are larger and less dense, if they consist of more fat than of protein. They are classified on the basis of electrophoresis and ultracentrifugation.

Chylomicrons: They are synthesized in the intestine. They carry triglycerides (fat) from the intestines to the liver, skeletal muscle, and to adipose tissue. Chylomicrons are, therefore, the molecules formed to mobilize dietary (exogenous) lipids. They consist of the highest quantity of lipid and lowest concentration (1%) of protein. The chylomicrons are the least in density and the largest in size among the lipoproteins. The predominant lipids of chylomicrons are triacylglycerols. The apolipoproteins that predominate before the chylomicrons enter the circulation include apoB-48 and apoA-I, apoA-II and apoA-IV. ApoB-48 combines only with chylomicrons.

Very low-density lipoproteins (VLDL): These are produced in the liver and the intestine. Very low-density lipoproteins (VLDL) carry (newly synthesized) triacylglycerol from the liver to adipose tissue. The dietary intake of both fat and carbohydrate, in excess of the needs of the body, leads to their conversion into triacylglycerols in the liver. These triacylglycerols are packaged into VLDLs and released into the circulation for delivery to the various tissues (primarily muscle and adipose tissue) for storage or production of energy through oxidation. VLDLs are, therefore, the molecules formed to transport endogenously derived triacylglycerols to extra-hepatic tissues. In addition to triacylglycerols, VLDLs contain some cholesterol and cholesteryl esters and the apoproteins, apoB-100, apoC-I, apoC-II, apoC-III and apoE. Like nascent chylomicrons, newly released VLDLs acquire apoCs and apoE from circulating HDLs.

Intermediate density lipoproteins (IDL):

Intermediate density lipoproteins (IDL) are intermediate between VLDL and LDL. They are not usually detectable in the blood.

IDLs are formed as triacylglycerols are removed from VLDLs. The fate of IDLs is either conversion to LDLs or direct uptake by the liver. Conversion of IDLs to LDLs occurs as more triacylglycerols are removed. The liver takes up IDLs after they have interacted with the LDL receptor to form a complex, which is endocytosed by the cell. For LDL receptors in the liver to recognize IDLs require the presence of both apoB-100 and apoE.

Low-density lipoprotein (LDL): They are formed from VLDL in the blood circulation. Low-density lipoproteins (LDL) carry endogenous cholesterol from the liver to cells of the body. LDLs are sometimes referred to as the "bad cholesterol" lipoprotein.

High-density lipoprotein (HDL): They are mostly synthesized in the liver. Three different fractions of HDL can be identified (1, 2, and 3) by ultracentrifugation. High-density

lipoproteins (HDL) collect cholesterol from the body's tissues, and bring it back to the liver where it can ultimately be excreted following conversion to bile acids. This function is referred to as reverse cholesterol transport (RCT). The role of HDLs in RCT represents the major atheroprotective (prevention of the development of atherosclerotic lesions in the vasculature) function of this class of lipoprotein. In addition to RCT, HDLs exert anti-inflammatory, antioxidant, and vasodilatory effects that together represent addition atheroprotective functions of HDLs. Evidence has also been generated that demonstrates that HDLs possess anti-apoptotic, anti-thrombotic, and anti-infectious properties. With respect to these various atheroprotective functions of HDLs, it is the small dense particles (referred to as HDL_3) that are the most beneficial. HDLs are sometimes referred to as the "good cholesterol" lipoprotein.

Density (g/mL)	**Class**	Diameter (nm)	% protein	% cholesterol	% phospholipid	% triacylglycerol
>1.063	HDL	5–15	33	30	29	4
1.019–1.063	LDL	18–28	25	50	21	8
1.006–1.019	IDL	25–50	18	29	22	31
0.95–1.006	VLDL	30–80	10	22	18	50
<0.95	Chylomicrons	100-1000	<2	8	7	84

Apolipoproteins

The protein components of lipoproteins are known as apolipoproteins or simply, apoproteins. **Apolipoproteins** are proteins that bind to lipids (oil-soluble substances such as fat and cholesterol) to form lipoproteins, which transport the lipids through the lymphatic and circulatory systems.

The lipid components of lipoproteins are not soluble in water; however, because of their detergent-like (amphipathic) properties, apolipoproteins and other amphipathic molecules (such as phospholipids) can surround the lipids, creating the lipoprotein particle that is itself water-soluble, and can thus be carried through water-based circulation (i.e., blood, lymph).

Apolipoproteins also serve as enzyme cofactors, receptor ligands, and lipid transfer carriers that regulate the metabolism of lipoproteins and their uptake in tissues.

They perform the following **functions:**

- Act as structural components of lipoproteins.

- They are enzyme coenzymes (C-II for lipoprotein lipase and A-I for lecithin-cholesterol acyltransferase)
- They are lipid transport proteins
- Recognize the cell membrane surface receptors; ie they are ligands for interaction with lipoprotein receptors in tissues (apoB100 and apoE for LDL-receptors, apoA-I for HDL receptors)

Classes

There are six major classes of apolipoproteins and several sub-classes:

- A (apo A-I, apo A-II, apo A-IV, and apo A-V)
- B (apo B48 and apo B100)
- C (apo C-I, apo C-II, apo C-III, and apo C-IV)
- D
- E
- H

Apo B is an integral apoprotein whereas the others are peripheral apoproteins.

APOLIPOPROTEIN A-1 and A-11	Apo A-I and Apo A-II are the main protein components of HDL cholesterol. HDL participates in reverse cholesterol transport, removing excess cholesterol from the tissues for removal by the liver. Apo A-I is non-atherogenic but increased levels of Apo-II appear to promote atherosclerosis by displacing Apo A-I in HDL and inhibiting reverse cholesterol transport.
APOLIPOPROTEIN B	Apo B is a component of LDL cholesterol and enables tissue cells to take up cholesterol. Elevated levels of Apo B indicate increased cardiovascular risk even when total and LDL cholesterol levels are within the normal range.
APOLIPOPROTEIN C-I1 and C-111	Apo C-II and Apo C-III have antagonistic excitatory and inhibitory effects on lipoprotein lipase, which breaks down lipoproteins and hydrolyses triglycerides in VLDL and chylomicrons for absorption into tissue cells. Apo C-II deficiency leads to hypertriglyceridaemia; genetic Apo C-III deficiency increases the rate of triglyceride clearance. Low Apo C-II and elevated Apo C-III levels are associated with a variety of diseases such as type 2 diabetes, hypertriglyceridaemia and hyperbilirubinaemia.
APOLIPOPROTEIN E	There are three similar isoforms of Apo E: Apo E2, E3 and E4 with E3 being the most common. Apo E has a variety of functions depending on which lipoprotein it is in. Apo E

deficiency gives rise to high cholesterol and triglyceride levels, promoting atherosclerosis. The polymorphism has been associated with diseases other than cardiovascular disease, for example E4 is implicated in Alzheimer's disease.

Metabolism of lipoproteins

Chylomicrons leave the intestine via the lymphatic system and enter the circulation at the left subclavian vein. In the bloodstream, chylomicrons acquire apoC-II and apoE from plasma HDLs. In the capillaries of adipose tissue and muscle, the fatty acids of chylomicrons are removed from the triacylglycerols by the action of lipoprotein lipase (LPL), which is found on the surface of the endothelial cells of the capillaries. The apoC-II in the chylomicrons activates LPL in the presence of phospholipid. The free fatty acids are then absorbed by the tissues and the glycerol backbone of the triacylglycerols is returned, via the blood, to the liver and kidneys. Glycerol is converted to the glycolytic intermediate dihydroxyacetone phosphate (DHAP). During the removal of fatty acids, a substantial portion of phospholipid, apoA and apoC is transferred to HDLs. The loss of apoC-II prevents LPL from further degrading the chylomicron remnants.

Chylomicron remnants, containing primarily cholesteryl esters, apoE and apoB-48, are then delivered to, and taken up by, the liver. The remnant particle must be of a sufficiently small size such that can pass through the fenestrated endothelial cells lining the hepatic sinusoids. Chylomicron remnants can then be taken up by hepatocytes via interaction with the LDL receptor which requires apoE. Chylomicron remnants may be further metabolized which increases apoE and lysophospholipid content allowing for transfer to LDL receptors or LDL receptor –related proteins (LRP) for hepatic uptake.

The fatty acid portion of **very low-density lipoproteins** (VLDLs) is released to adipose tissue and muscle in the same way as for chylomicrons, through the action of lipoprotein lipase. The action of lipoprotein lipase coupled to a loss of certain apoproteins (the apoCs) converts VLDLs to intermediate density lipoproteins (IDLs), also termed VLDL remnants. The apoCs are transferred to HDLs. The predominant remaining proteins are apoB-100 and apoE. Further loss of triacylglycerols converts IDLs to LDLs.

The exclusive apolipoprotein of **low-density lipoproteins** (LDLs) is apoB-100. Apolipoprotein –B100 is responsible for the recognition of LDL receptor sites. LDLs are taken up by cells via LDL receptor-mediated endocytosis. The uptake of LDLs occurs predominantly in liver (75%), adrenals and adipose tissue. As with IDLs, the interaction of LDLs with LDL receptors requires the presence of apoB-100. The endocytosed membrane vesicles (endosomes) fuse with lysosomes, in which the apoproteins are degraded and the cholesterol esters are hydrolyzed to yield free cholesterol. The cholesterol is then incorporated into the plasma membranes as necessary. Excess intracellular cholesterol is

re-esterified by acyl-CoA-cholesterol acyltransferase (**ACAT**), for intracellular storage. The activity of ACAT is enhanced by the presence of intracellular cholesterol. A defect in LDL receptors result in the elevation of plasma LDL and hence plasma cholestrol, but plasma triacylglycerol remains the same.

The consumption of alcohol is associated with either a protective or a negative effect on the level of circulating LDL. Low level alcohol consumption, particularly red wines which contain the antioxidant resveratrol, appears to be beneficial with respect to cardiovascular health. One major effect of resveratrol in the blood is the prevention of oxidation of LDLs, (forming oxLDL). Oxidized LDLs contribute significantly to the development of athersclerosis. Conversely excess alcohol consumption is associated with the development of fatty liver which in turn impairs the ability of the liver to take up LDL via the LDL receptor resulting in increased LDL in the circulation.

High-density lipoproteins (HDLs) are synthesized in the liver as discoid particles. They contain free cholesterol and phospholipids (mostly lecithin) and apoproteins A, C11, E, etc. The plasma enzyme lecithin-cholesterol acyl transferase (LCAT) catalyzes the esterification of free cholesterol (by fatty acid of lecithin) present in the extra hepatic tissues and transfers to the HDL. Apoprotein A promotes the activity of LCAT. HDL also accepts free cholesterol from other lipoproteins in circulation and cell membrane of peripheral tissues. Due to the addition of cholesterol, HDL particles become spherical.

The HDL particles with cholesteryl ester trapped inside, enter the hepatocytes by a receptor- mediated endocytosis. In the liver, the cholesteryl esters are degraded to cholesterol which is utilized for the synthesis of bile acids and lipoproteins or excreted into bile (as cholesterol).

Cholesteyl ester transfer protein (CETP) is synthesized in the liver and it facilitates the transfer of cholesteryl esters from HDL to VLDL or LDL in exchange for triglyceride (TG).

Clinical Significances of Lipoprotein Metabolism

Fortunately, few individuals carry the inherited defects in lipoprotein metabolism that lead to hyper- or hypolipoproteinemias . Persons suffering from diabetes mellitus, hypothyroidism and kidney disease often exhibit abnormal lipoprotein metabolism as a result of secondary effects of their disorders. For example, because lipoprotein lipase (LPL) synthesis is regulated by insulin, LPL deficiencies leading to Type I hyperlipoproteinemia may occur as a secondary outcome of diabetes mellitus. Additionally, insulin and thyroid hormones positively affect hepatic LDL-receptor interactions; therefore, the hypercholesterolemia and increased risk of atherosclerosis associated with uncontrolled diabetes or hypothyroidism is likely due to decreased hepatic LDL uptake and metabolism.

Of the many disorders of lipoprotein metabolism, familial hypercholesterolemia (FH) may be the most prevalent in the general population. Heterozygosity at the FH locus occurs in 1:500 individuals, whereas, homozygosity is observed in 1:1,000,000 individuals. FH is an

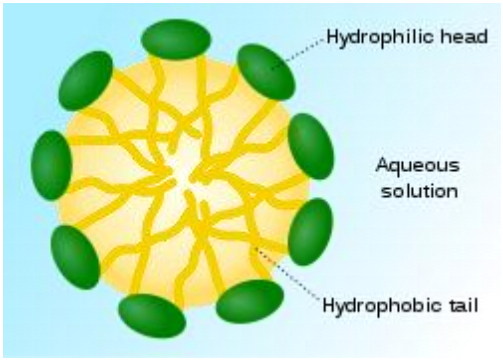
inherited disorder comprising four different classes of mutation in the LDL receptor gene. The class 1 defect (the most common) results in a complete loss of receptor synthesis. The class 2 defect results in the synthesis of a receptor protein that is not properly processed in the Golgi apparatus and therefore is not transported to the plasma membrane. The class 3 defect results in an LDL receptor that is incapable of binding LDLs. The class 4 defect results in receptors that bind LDLs but do not cluster in coated pits and are, therefore, not internalized.

FH sufferers may be either heterozygous or homologous for a particular mutation in the receptor gene. Homozygotes exhibit grossly elevated serum cholesterol (primarily in LDLs). The elevated levels of LDLs result in their phagocytosis by macrophages. These lipid-laden phagocytic cells tend to deposit within the skin and tendons, leading to xanthomas. A greater complication results from cholesterol deposition within the arteries, leading to atherosclerosis, the major contributing factor of nearly all cardiovascular diseases.

Lipid micelles

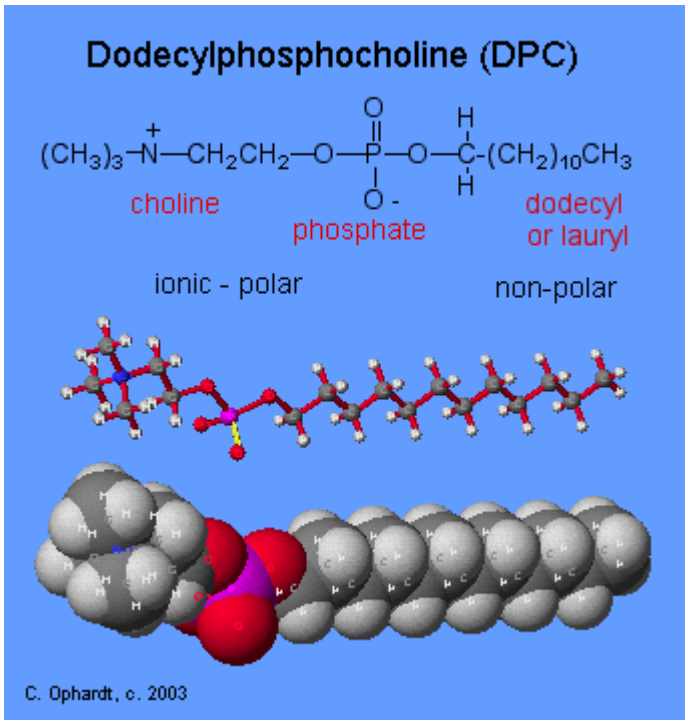
A **micelle** is an aggregate of surfactant molecules dispersed in a liquid colloid. A colloid is a type of mixture in which one substance is evenly dispersed throughout another. There's generally two phases - the **dispersed** or internal phase and the **continuous phase**. A typical micelle in aqueous solution forms an aggregate with the hydrophilic "head" regions in contact with surrounding solvent, sequestering the hydrophobic tail regions in the centre. The hydrophobic effects of the tail region of these molecules result in the formation of spherical structures or micelles.

The hydrophobic tails are concentrated at the heart of the sphere where they form a **hydrophobic core** (**yellow**) and are held together by numerous van der Waals interactions. Simultaneously the hydrated polar heads form an **external envelope** which strongly interacts with the surrounding water and also hinders physical contacts between the water and the hydrophobic core. This type of micelle is known as a normal phase micelle (oil-in-water micelle).



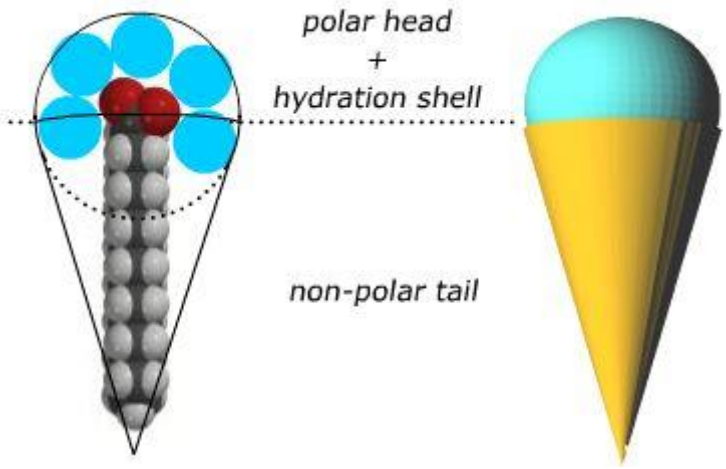
Scheme of a **micelle** formed by phospholipids in an aqueous solution.

A micelle is formed when a variety of molecules including soaps and detergents are added to water. The molecule may be a fatty acid, a salt of a fatty acid (soap), phospholipids, or other similar molecules. The molecule must have a strongly polar "head" and a non-polar hydrocarbon chain "tail". When this type of molecule is added to water, the non-polar tails of the molecules clump into the center of a ball like structure called a micelle, because they are hydrophobic or "water hating". The polar head of the molecule presents itself for interaction with the water molecules on the outside of the micelle.



Structure of Dodecylphosphocholine (DPC): DPC is a synthetic phosphodiester. The choline, which contains a quaternary amine with a positive charge, and the phosphate are ionic and polar. The dodecyl part is the non-polar hydrocarbon chain.

As illustrated below, amphiphilic molecules with only one long alkyl chain and a hydration shell surrounding the polar head may form roughly **conical structures.** This is mostly the case for fatty acids and their soaps.



Steric occupancy of soap (palmitate).

Micelles are stable structures. Their size and the number of amphiphilic molecules they contain are determined by the length of the alkyl chains of these molecules. Micelles are tiny particles which remain indefinitely in suspension in water. Their non-polar cores can capture and solvate greasy substances, hence the **cleaning power** of soaps (and related molecules).

Micelles are approximately spherical in shape. Spherical micelles contain less than 100 surfactant molecules. Other phases, including shapes such as ellipsoids, cylinders, and bilayers are also possible. The shape and size of a micelle is a function of the molecular geometry of its surfactant molecules and solution conditions such as surfactant concentration, temperature, pH, and ionic strength. The process of forming micelles is known as micellization.

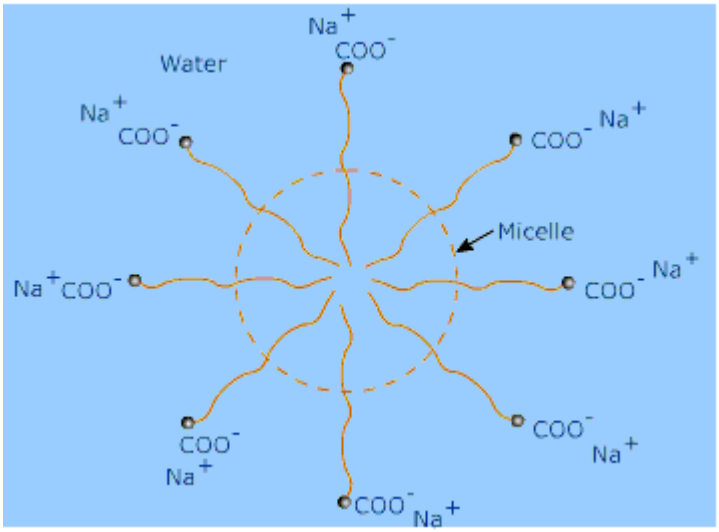
Micelles only form when the concentration of surfactant is greater than the critical micelle concentration (CMC), and the temperature of the system is greater than the critical micelle temperature, or Krafft temperature.

Mechanism of Micelle Formation

Micelles are formed by specific molecules which have lyophilic as well as lyophobic ends. Ordinary soap which contains sodium stearate ($C_{17}H_{35}COONa$) forms micelle in water. The stearate ion has a long hydrocarbon end that is hydrophobic (because it is nonpolar) and a polar carboxyl group (COO^-) that is hydrophilic.

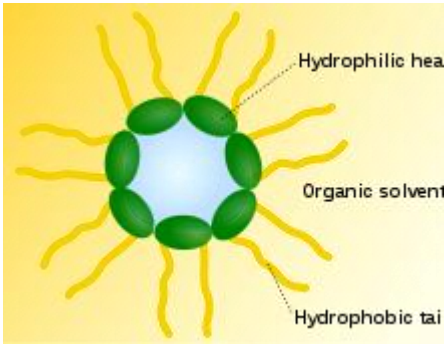
When the concentration of sodium stearate is below its CMC, then it behaves as a normal electrolyte and ionizes to give Na^+ and $C_{17}H_{35}COO^-$ ions. As the concentration exceeds the

CMC, the hydrophobic ends start receding away from the solvent and approach each other. However, the polar COO^- parts interact with water. This leads to the formation of a cluster having the dimensions of colloid particles. In each cluster a large number of stearate groups clump together in a spherical manner such that the hydrocarbon parts interact with one another and the COO^- groups remain projected in water.



Inverse/reverse micelles

In a non-polar solvent, it is the exposure of the hydrophilic head groups to the surrounding solvent that is energetically unfavourable, giving rise to a water-in-oil system. In this case the hydrophilic groups are sequestered in the micelle core and the hydrophobic groups extend away from the centre. These inverse micelles are proportionally less likely to form on increasing headgroup charge, since hydrophilic sequestration would create highly unfavorable electrostatic interactions.



Scheme of an **inverse micelle** formed by phospholipids in an organic solvent.

Uses of micelles

When surfactants are present above the Critical micelle concentration (CMC), they can act as emulsifiers, that will allow a compound that is normally insoluble (in the solvent being used) to dissolve. This occurs because the insoluble species can be incorporated into the micelle core, which is itself solubilized in the bulk solvent by virtue of the head groups' favorable interactions with solvent species. In other words, a micellar solution is unique in that it allows non-polar solutes to dissolve in a polar medium.

The most common example of this phenomenon is detergents, which clean poorly soluble lipophilic material (such as oils and waxes) that cannot be removed by water alone. Detergents also clean by lowering the surface tension of water, making it easier to remove material from a surface. In detergent mixes - like body washes and shampoos - the micelles help to create the viscosity of the product and emulsify small amounts of oils (like fragrance, essential, or specialty oils).

Again in oil-in-water lotions and creams, micelles provide emulsification. The oil attaches to the hydrophobic tails inside the micelle and the water attaches to the hydrophilic heads on the outside. Without micelles, there would be no emulsification.

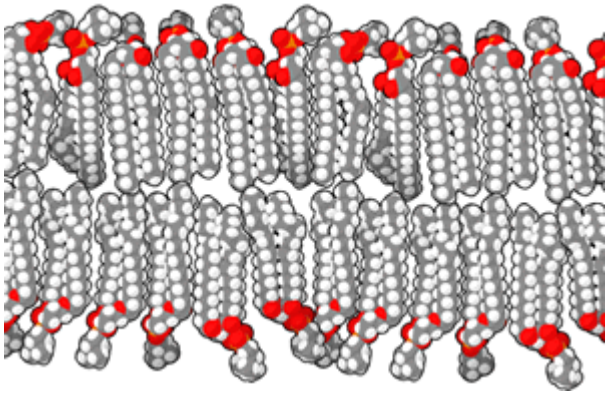
Micelle formation is also essential for the absorption of fat-soluble vitamins and complicated lipids within the human body. Bile salts formed in the liver and secreted by the gall bladder allow micelles of fatty acids to form. This allows the absorption of complicated lipids (e.g., lecithin) and lipid soluble vitamins (A, D, E and K) within the micelle by the small intestine.

Lipid bilayer

A lipid bilayer is a sheet of lipids, two-molecules thick, arranged so that the hydrophilic phosphate heads point “out” to the water on either side of the bilayer and the hydrophobic tails point “in” to the core of the bilayer. This arrangement results in two “leaflets” which are each a single molecular layer. Lipids self-assemble into this structure because of the hydrophobic effect, which creates an energetically unfavorable interaction between the hydrophobic lipid tails and the surrounding water. Thus, a lipid bilayer is typically held together by entirely non-covalent forces that do not involve formation of chemical bonds between individual molecules.

The lipid bilayer is a thin membrane made of two layers of lipid molecules. These membranes are flat sheets that form a continuous barrier around cells. The cell membrane of almost all living organisms and many viruses are made of a lipid bilayer, as are the membranes surrounding the cell nucleus and other sub-cellular structures. The lipid bilayer is the barrier that keeps ions, proteins and other molecules where they are needed and

prevents them from diffusing into areas where they should not be. Lipid bilayers are ideally suited to this role because, even though they are only a few nanometers in width, they are impermeable to most water-soluble (hydrophilic) molecules. Bilayers are particularly impermeable to ions, which allow cells to regulate salt concentrations and pH by pumping ions across their membranes using proteins called ion pumps.



This fluid lipid bilayer cross section is made up entirely of phosphatidylcholine.

Natural bilayers are usually made mostly of phospholipids, which have a hydrophilic head and two hydrophobic tails. When phospholipids are exposed to water, they arrange themselves into a two-layered sheet (a bilayer) with all of their tails pointing toward the center of the sheet. The center of this bilayer contains almost no water and also excludes molecules like sugars or salts that dissolve in water but not in oil. This assembly process is similar to the coalescing of oil droplets in water and is driven by the same force, called the hydrophobic effect.

Lipid tails can also affect membrane properties, for instance by determining the phase of the bilayer. The bilayer can adopt a solid gel phase state at lower temperatures but undergo phase transition to a fluid state at higher temperatures. The packing of lipids within the bilayer also affects its mechanical properties, including its resistance to stretching and bending.

Biological membranes typically include several types of lipids other than phospholipids. A particularly important example in animal cells is cholesterol, which helps strengthen the bilayer and decrease its permeability. Cholesterol also helps regulate the activity of certain integral membrane proteins. Integral membrane proteins function when incorporated into a lipid bilayer. Because bilayers define the boundaries of the cell and its compartments, these membrane proteins are involved in many intra- and inter-cellular signaling processes. Certain kinds of membrane proteins are involved in the process of fusing two bilayers together. This fusion allows the joining of two distinct structures as in the fertilization of an egg by sperm or the entry of a virus into a cell.

Asymmetry of the bilayer

In many naturally occurring bilayers, the compositions of the inner and outer membrane leaflets are different. In human red blood cells, the inner (cytoplasmic) leaflet is largely composed of phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol and its phosphorylated derivatives. By contrast, the outer (extracellular) leaflet is based on phosphatidylcholine, sphingomyelin and a variety of glycolipids. The biological functions of lipid asymmetry are imperfectly understood, although it is clear that it is used in several different situations. For example, when a cell undergoes apoptosis, the phosphatidylserine — normally localised to the cytoplasmic leaflet — is transferred to the outer surface: there it is recognised by a macrophage which then actively scavenges the dying cell.

Phases and phase transitions

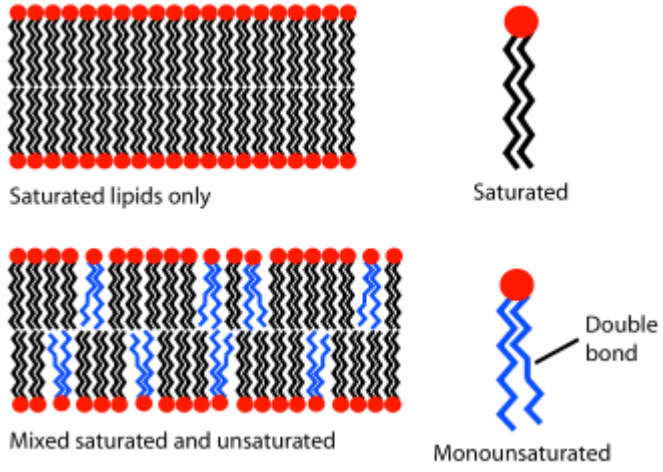


Diagram showing the effect of unsaturated lipids on a bilayer. The lipids with an unsaturated tail (blue) disrupt the packing of those with only saturated tails (black). The resulting bilayer has more free space and is consequently more permeable to water and other small molecules.

Lipid bilayer phase behavior

At a given temperature a lipid bilayer can exist in either a liquid or a gel (solid) phase. All lipids have a characteristic temperature at which they transition (melt) from the gel to liquid phase. In both phases the lipid molecules are prevented from flip-flopping across the bilayer, but in liquid phase bilayers a given lipid will exchange locations with its neighbor millions of times a second. This random walk exchange allows lipid to diffuse and thus wander across the surface of the membrane. Unlike liquid phase bilayers, the lipids in a gel phase bilayer are locked in place.

The phase behavior of lipid bilayers is largely determined by the strength of the attractive Van der Waals interactions between adjacent lipid molecules. Longer tailed lipids have more area over which to interact, increasing the strength of this interaction and consequently decreasing the lipid mobility. Thus, at a given temperature, a short-tailed lipid will be more fluid than an otherwise identical long-tailed lipid. Transition temperature can also be affected by the degree of unsaturation of the lipid tails. An unsaturated double bond can produce a kink in the alkane chain, disrupting the lipid packing. This disruption creates extra free space within the bilayer which allows additional flexibility in the adjacent chains. An example of this effect can be noted in everyday life as butter, which has large percentage saturated fats, is solid at room temperature while vegetable oil, which is mostly unsaturated, is liquid.

Most natural membranes are a complex mixture of different lipid molecules. If some of the components are liquid at a given temperature while others are in the gel phase, the two phases can coexist in spatially separated regions, rather like an iceberg floating in the ocean. This phase separation plays a critical role in biochemical phenomena because membrane components such as proteins can partition into one or the other phase and thus be locally concentrated or activated. One particularly important component of many mixed phase systems is cholesterol, which modulates bilayer permeability, mechanical strength and biochemical interactions.

While lipid tails primarily modulate bilayer phase behavior, it is the headgroup that determines the bilayer surface chemistry. Most natural bilayers are composed primarily of phospholipids, although sphingolipids such as sphingomyelin and sterols such as cholesterol are also important components. Of the phospholipids, the most common headgroup is phosphatidylcholine (PC), accounting for about half the phospholipids in most mammalian cells.

Other headgroups are also present to varying degrees and can include phosphatidylserine (PS) phosphatidylethanolamine (PE) and phosphatidylglycerol (PG). These alternate headgroups often confer specific biological functionality that is highly context-dependent. For instance, PS presence on the extracellular membrane face of erythrocytes is a marker of cell apoptosis, whereas PS in growth plate vesicles is necessary for the nucleation of hydroxyapatite crystals and subsequent bone mineralization.

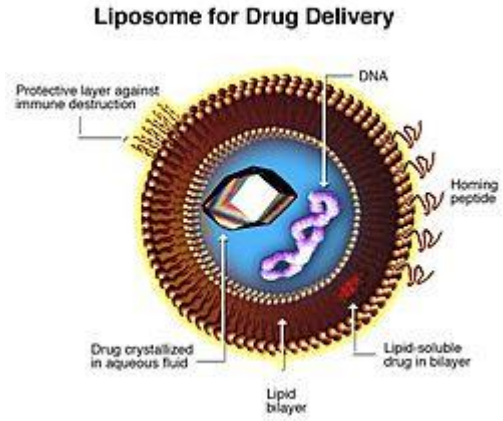
Biological roles of lipid bilayer

The primary role of the lipid bilayer in biology is to separate aqueous compartments from their surroundings. Without some form of barrier delineating “self” from “non-self” it is difficult to even define the concept of an organism or of life. This barrier takes the form of a lipid bilayer in all known life forms except for a few species of archaea which utilize a

specially adapted lipid monolayer. The partitioning ability of the lipid bilayer is based on the fact that hydrophilic molecules cannot easily cross the hydrophobic bilayer core.

Lipid bilayers are also involved in signal transduction through their role as the home of integral membrane proteins. This is an extremely broad and important class of biomolecule. Some of these proteins are linked to the exterior of the cell membrane. An example of this is the CD59 protein, which identifies cells as “self” and thus inhibits their destruction by the immune system. The HIV virus evades the immune system in part by grafting these proteins from the host membrane onto its own surface. Alternatively, some membrane proteins penetrate all the way through the bilayer and serve to relay individual signal events from the outside to the inside of the cell. The most common class of this type of protein is the G protein-coupled receptor (GPCR). GPCRs are responsible for much of the cell’s ability to sense its surroundings and, because of this important role; approximately 40% of all modern drugs are targeted at GPCRs.

Liposomes



The name liposome is derived from two Greek words: 'LIPO' meaning fat and 'Soma' meaning body. Liposomes are artificially prepared vesicles made of lipid bilayer. They are composite structures made of phospholipids and may contain small amounts of other molecules. Though liposomes can vary in size from low micrometer range to tens of micrometers, unilamellar liposomes, as pictured here, are typically in the lower size range with various targeting ligands attached to their surface allowing for their surface-attachment and accumulation in pathological areas for treatment of disease. Liposomes can be composed of naturally derived phospholipids with mixed lipid chains or other surfactants. Liposomes should not be confused with micelles and reverse micelles composed of monolayers.

Applications

Liposomes are used for drug delivery due to their unique properties. A liposome encapsulates a region of aqueous solution inside a hydrophobic membrane; since dissolved hydrophilic solutes cannot readily pass through the lipids. Hydrophobic chemicals can be dissolved into the membrane, and in this way, liposome can carry both hydrophobic molecules and hydrophilic molecules. To deliver the molecules to sites of action, the lipid bilayer can fuse with other bilayers such as the cell membrane, thus delivering the liposome contents. By making liposomes in a solution of DNA or drugs (which would normally be unable to diffuse through the membrane) they can be (indiscriminately) delivered past the lipid bilayer. There are three types of liposomes - MLV (multilamellar vesicles) SUV (Small Unilamellar Vesicles) and LUV (Large Unilamellar Vesicles). These are used to deliver different types of drugs.

Liposomes can also be designed to deliver drugs in other ways. Liposomes that contain low (or high) pH can be constructed such that dissolved aqueous drugs will be charged in solution (i.e., the pH is outside the drug's pI range). As the pH naturally neutralizes within the liposome (protons can pass through some membranes), the drug will also be neutralized, allowing it to freely pass through a membrane. These liposomes work to deliver drug by diffusion rather than by direct cell fusion.

A similar approach can be exploited in the biodetoxification of drugs by injecting empty liposomes with a transmembrane pH gradient. In this case the vesicles act as sinks to scavenge the drug in the blood circulation and prevent its toxic effect. Liposomes can also be used for transformation or transfection of DNA into a host cell in a process known as lipofection.

Liposomes are presently being cleverly implemented for the specific oral delivery of certain dietary and nutritional supplements. A very small number of dietary and nutritional supplement companies are currently pioneering the benefits of this unique science towards this new application. This new direction and employment of Liposome science is in part due to the low absorption and bioavailability rates of traditional oral dietary and nutritional tablets and capsules. Therefore the natural encapsulation of lypophilic and hydrophilic nutrients within Liposomes has made for a very effective method of bypassing the destructive elements of the gastric system and aiding the encapsulated nutrient to be delivered to the cells and tissues.

Targeting cancer

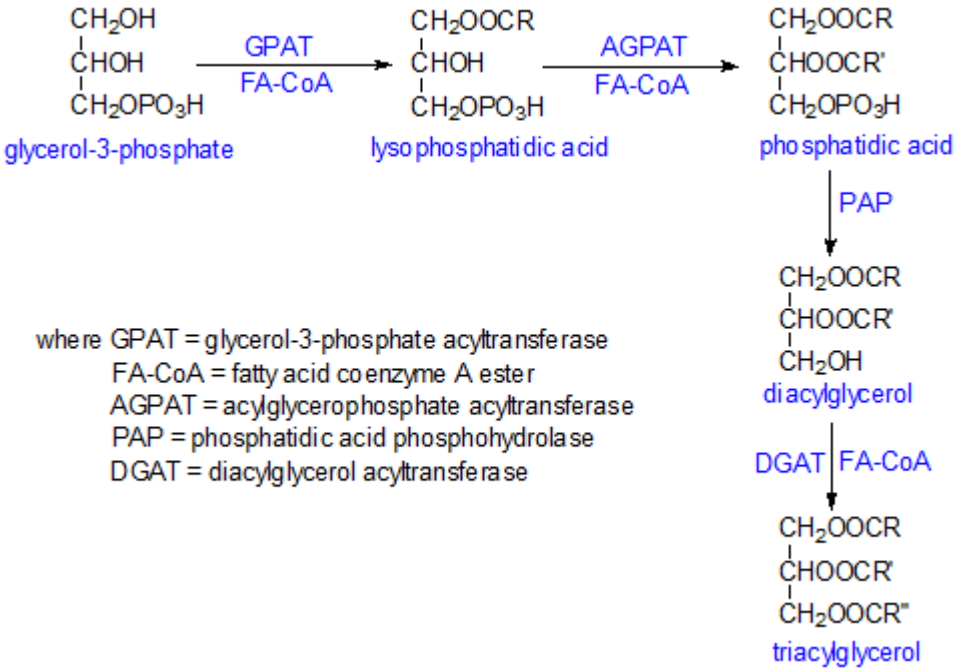
Another interesting property of liposomes is their natural ability to target cancer. The endothelial wall of all healthy human blood vessels is encapsulated by endothelial cells that are bound together by tight junctions. These tight junctions stop any large particles in the blood from leaking out of the vessel. Tumour vessels do not contain the same level of seal

between cells and are diagnostically leaky. This ability is known as the Enhanced Permeability and Retention effect. Liposomes of certain sizes, typically less than 200 nm, can rapidly enter tumour sites from the blood, but are kept in the bloodstream by the endothelial wall in healthy tissue vasculature. Anti-cancer drugs such as Doxorubicin (Doxil), Camptothecin and Daunorubicin (Daunoxome) are currently being marketed in liposome delivery systems.

Biosynthesis of triacylglycerol

All eukaryotic organisms and even a few prokaryotes have the ability to synthesize triacylglycerols. Many cell types and organs have the ability to synthesize triacylglycerols, but in animals the liver and intestines are most active, although most of the body stores of this lipid are in adipose tissue. Within all cell types, even those of the brain, triacylglycerols are stored as cytoplasmic '**lipid droplets**' (also termed 'fat globules', 'oil bodies', 'lipid particles', 'adiposomes', etc) enclosed by a monolayer of phospholipids and hydrophobic proteins.

Two main biosynthetic pathways are known, the *sn*-glycerol-3-phosphate pathway, which predominates in liver and adipose tissue, and a monoacylglycerol pathway in the intestines. The most important route to triacylglycerol biosynthesis is the *sn*-glycerol-3-phosphate or **Kennedy pathway** illustrated below, first described by Professor Eugene Kennedy and colleagues in the 1950s, by means of which more than 90% of liver triacylglycerols are produced.



In the *sn*-glycerol-3-phosphate or α-glycerophosphate pathway, the main source of the glycerol backbone has long been believed to be *sn*-glycerol-3-phosphate produced by the catabolism of glucose (glycolysis) or to a lesser extent by the action of the enzyme glycerol kinase on free glycerol. However, there is increasing evidence that a significant proportion of the glycerol is produced *de novo* by a process known as glyceroneogenesis via pyruvate. Indeed, this may be the main source in adipose tissue.

Subsequent reactions occur in the endoplasmic reticulum. First, the precursor *sn*-glycerol-3-phosphate is esterified by a fatty acid coenzyme A ester in a reaction catalysed by a glycerol-3-phosphate acyltransferase (GPAT) at position *sn*-1 to form lysophosphatidic acid, and this is in turn acylated by an acylglycerophosphate acyltransferase in position *sn*-2 to form a key intermediate in the biosynthesis of all glycerolipids - **phosphatidic acid**.

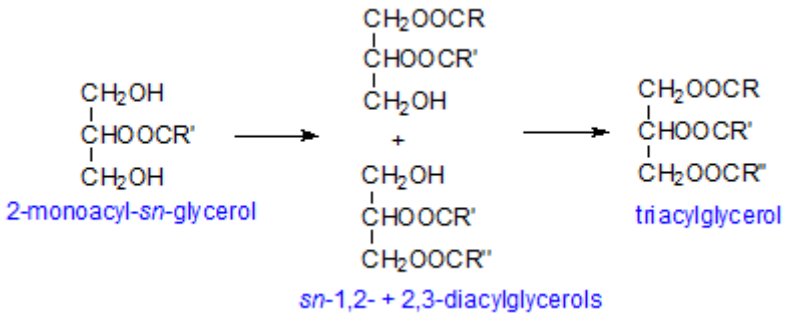
The phosphate group is removed by an enzyme (or family of enzymes) phosphatidic acid phosphohydrolase (PAP or 'phosphatidate phosphatase' or 'lipid phosphate phosphatase'). PAP is also important as it produces diacylglycerols as essential intermediates in the biosynthesis of phosphatidylcholine and phosphatidylethanolamine. Much of the phosphatase activity leading to triacylglycerol biosynthesis resides in three related cytoplasmic proteins, termed lipin-1, lipin-2, and lipin-3. The lipins are tissue specific, but lipin-1 (PAP1) accounts for all the PAP activity in adipose tissue and skeletal muscle. While it occurs mainly in the cytosolic compartment of cells, it is translocated to the endoplasmic reticulum in response to elevated levels of fatty acids within cells. Lipin-1 activity requires Mg^{2+} ions and is inhibited by *N*-ethylmaleimide. Lipin 2 is a similar phosphatidate phosphohydrolase, which is present in liver and brain and is regulated dynamically by fasting and obesity (in mice), while lipin 3 is found in the gastrointestinal tract and liver.

Finally, the resultant 1, 2-diacyl-*sn*-glycerol is acylated by a diacylglycerol acyltransferase (DGAT) to form the triacyl-*sn*-glycerol. DGAT is the dedicated triacylglycerol-forming enzyme, and this is seen as a target for pharmaceutical intervention in obesity and attendant ailments.

In fact, there are two DGAT enzymes, skin which are structurally and functionally distinct. DGAT1 is expressed in skeletal muscle, and intestine, with lower levels of expression in liver and adipose tissue. It is the only one present in the epithelial cells that synthesize milk fat in the mammary gland. DGAT2 is the main form of the enzyme in hepatocytes and adipocytes. Both enzymes are important modulators of energy metabolism, although

DGAT2 appears to be especially important in controlling the homeostasis of triacylglycerols *in vivo*.

In the enterocytes of intestines after a meal, up to 75% of the triacylglycerols are formed via a monoacylglycerol pathway. 2-Monoacyl-*sn*-glycerols and free fatty acids released from dietary triacylglycerols by the action of pancreatic lipase within the intestines are taken up by the enterocytes. There, the monoacylglycerols are first acylated by an acyl coenzyme A: monoacylglycerol acyltransferase with formation of *sn*-1, 2-diacylglycerols mainly as the first intermediate in the process, though *sn*-2, 3-diacylglycerols are also produced, and then by acyl coenzyme A: diacylglycerol acyltransferase (DGAT1) to form triacylglycerols. DGAT1 can also acylate monoacylglycerols.

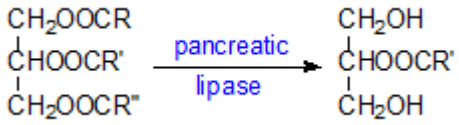


While triacylglycerols are essential for normal physiology, an excessive accumulation in human adipose tissue and other organs results in obesity and other health problems, including insulin resistance, steatohepatitis and cardiomyopathy. Accordingly, there is considerable pharmaceutical interest in drugs that affect triacylglycerol biosynthesis and metabolism.

Triacylglycerol Metabolism (degradation) in the Intestines, Liver and Mammary Gland

Fat comprises about 40% of the energy intake in the human diet in Western countries, and a high proportion of this is triacylglycerols. The process of fat digestion is begun in the stomach by acid-stable gastric or lingual lipases, the extent of which depending on species but may be important for efficient emulsification. However, this is insignificant in quantitative terms in comparison to the reaction with the colipase-dependent pancreatic lipase, which occurs in the duodenum. Entry of triacylglycerol degradation products into the duodenum stimulates synthesis of the hormone cholecystokinin and causes the gall bladder to release **bile acids**, which are strong detergents and act to emulsify the hydrophobic triacylglycerols so increasing

the available surface area. In turn, cholecystokinin stimulates the release of the hydrolytic enzyme pancreatic lipase. The process of hydrolysis is regiospecific and results in the release of the fatty acids from the 1(3) positions of the triacylglycerols and formation of 2-monoacyl-*sn*-glycerols. Isomerization of the latter to 1(3)-monoacyl-*sn*-glycerols occurs to some extent, and these can be degraded completely to glycerol and free acids.

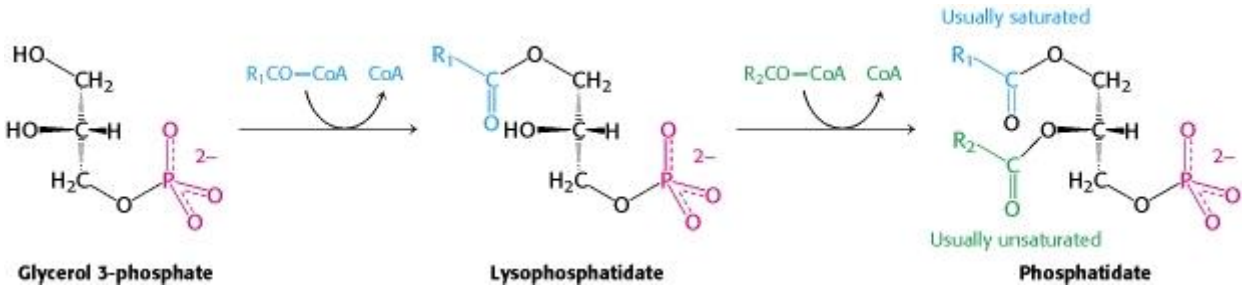


The free fatty acids and 2-monoacyl-*sn*-glycerols are rapidly taken up by the intestinal cells, via specific carrier molecules but possibly also by passive diffusion, and they are esterified into triacylglycerols as described by the biosynthetic pathway.

There is evidence that the regiospecific structure of dietary triglycerides has an effect on the uptake of particular fatty acids and may influence further the lipid metabolism in humans. In particular, incorporation of palmitic acid into the position *sn*-2 of milk fat may be of benefit to the human infant (as a source of energy for growth and development), although it increases the atherogenic potential for adults.

Synthesis of phospholipids

The first step in the synthesis of both phospholipids for membranes and triacylglycerols for energy storage is the synthesis of *phosphatidate* (diacylglycerol 3-phosphate). In mammalian cells, phosphatidate is synthesized in the endoplasmic reticulum and the outer mitochondrial membrane. It is formed by the addition of two fatty acids to *glycerol 3-phosphate,* which in turn is formed primarily by the reduction of dihydroxyacetone phosphate, a glycolytic intermediate, and to a lesser extent by the phosphorylation of glycerol. Glycerol 3-phosphate is acylated by acyl CoA to form *lysophosphatidate*, which is again acylated by acyl CoA to yield phosphatidate.

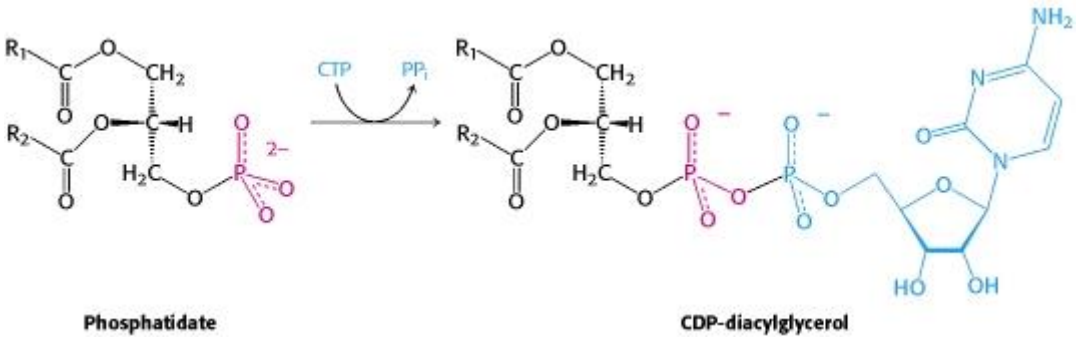


These acylations are catalyzed by *glycerol phosphate acyltransferase.* In most phosphatidates, the fatty acyl chain attached to the C-1 atom is saturated, whereas the one attached to the C-2 atom is unsaturated.

The pathways diverge at phosphatidate. Phospholipid synthesis requires the combination of a diacylglyceride with an alcohol. As in most anabolic reactions, one of the components must be activated. In this case, either of the two components may be activated, depending on the source of the reactants.

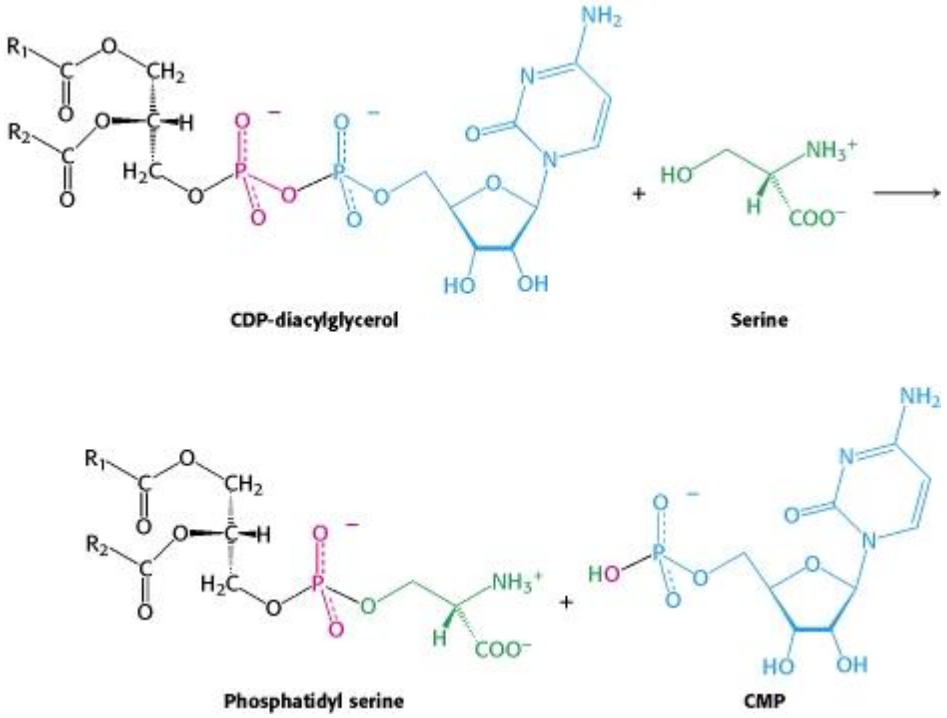
Synthesis from an Activated Diacylglycerol

The de novo pathway starts with the reaction of phosphatidate with cytidine triphosphate (CTP) to form *cytidine diphosphodiacylglycerol (CDP-diacylglycerol).* This reaction, like those of many biosyntheses, is driven forward by the hydrolysis of pyrophosphate.



Structure of CDP-Diacylglycerol. A key intermediate in the synthesis of phospholipids consists of phosphatidate and CMP joined by a pyrophosphate linkage.

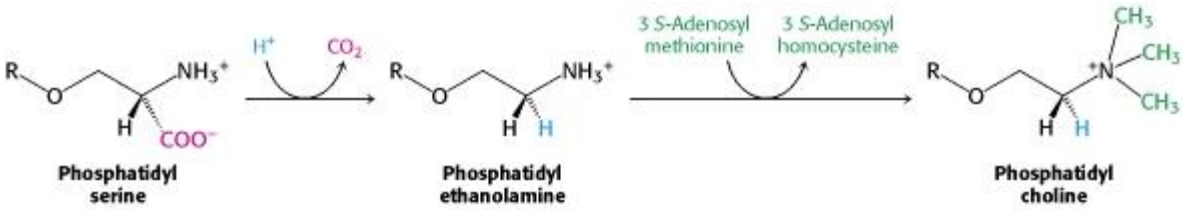
The activated phosphatidyl unit then reacts with the hydroxyl group of an alcohol to form a phosphodiester linkage. If the alcohol is serine, the products are *phosphatidyl serine* and cytidine monophosphate (CMP).



Likewise, phosphatidyl inositol is formed by the transfer of a diacylglycerol phosphate unit from CDP-diacylglycerol to inositol. Subsequent phosphorylations catalyzed by specific kinases lead to the synthesis of *phosphatidyl inositol 4,5-bisphosphate,* an important molecule in signal transduction.

The fatty acid components of a phospholipid may vary, and thus phosphatidyl serine, as well as most other phospholipids, represents a class of molecules rather than a single species. As a result, a single mammalian cell may contain thousands of distinct phospholipids. Phosphatidyl inositol is unusual in that it has a nearly fixed fatty acid composition. Stearic acid usually occupies the C-1 position and arachidonic acid the C-2 position.

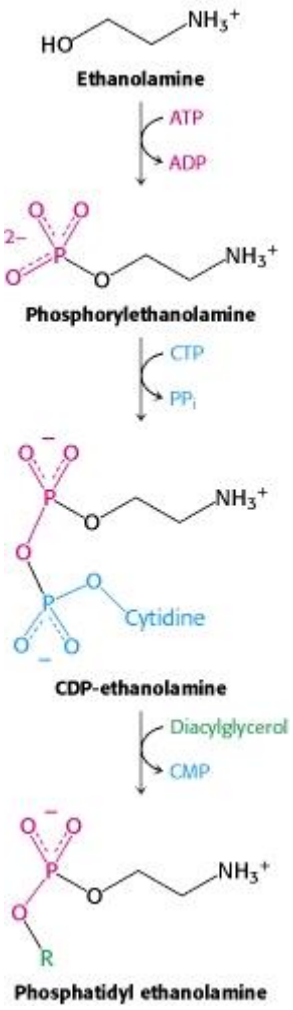
In bacteria, the decarboxylation of phosphatidyl serine by a pyridoxal phosphate-dependent enzyme yields *phosphatidyl ethanolamine,* another common phospholipid. The amino group of this phosphoglyceride is then methylated three times to form *phosphatidyl choline.* S-*Adenosylmethionine* is the methyl donor.



In mammals, phosphatidyl ethanolamine can be formed from phosphatidyl serine by the enzyme-catalyzed exchange of ethanolamine for the serine moiety of the phospholipid.

Synthesis from an Activated Alcohol

In mammals, phosphatidyl ethanolamine can also be synthesized from ethanolamine through the formation of CDP-ethanolamine. In this case, the alcohol ethanolamine is phosphorylated by ATP to form the precursor, *phosphorylethanolamine.* This precursor then reacts with CTP to form the activated alcohol, *CDP-ethanolamine.* The phosphorylethanolamine unit of CDP-ethanolamine is then transferred to a diacylglycerol to form *phosphatidyl ethanolamine.*



In mammals, a pathway that utilizes choline obtained from the diet ends in the synthesis of phosphatidyl choline, the most common phospholipid in these organisms. In this case, choline is activated in a series of reactions analogous to those in the activation of ethanolamine. Interestingly, the liver possesses an enzyme, *phosphatidyl ethanolamine methyltransferase,* that synthesizes phosphatidyl choline from phosphatidyl ethanolamine, through the successive methylation of ethanolamine. Thus, phosphatidyl choline can be produced by two distinct pathways, ensuring that this phospholipid can be synthesized even if the components for one pathway are in limited supply.

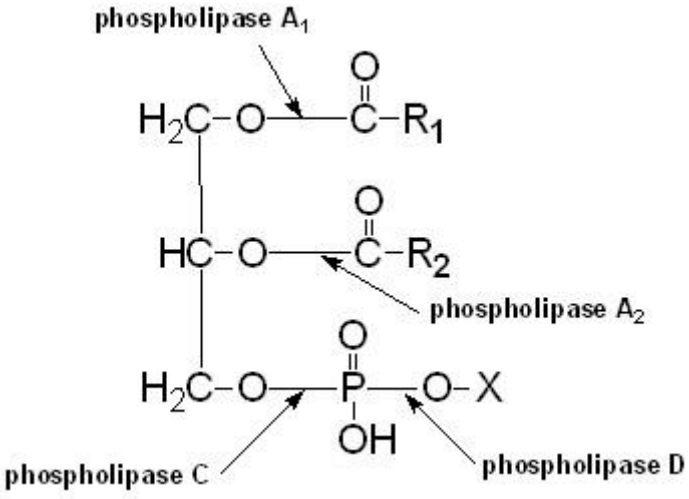
Degradation of phospholipids

Phospholipids are degraded by phospholipases which cleave the phosphodiester bonds. Phospholipase A_1 specifically cleaves the fatty acid at position C_1 of phospholipids resulting in lysophospholipid. Lysophospholipid can further be acted upon by phospholipase B to remove the second acyl group at position C_2.

Phospholipase A_2 hydrolyses the fatty acid at C_2 position of phospholipids. This enzyme is found in many tissues and pancreatic juice.

Phospholipase C specifically cleaves the bond between phosphate and glycerol of phospholipids. This enzyme is present in lysosome of hepatocytes.

Phospholipase D hydrolyses and removes the nitrogenous base from phospholipids. The degraded products of phospholipids enter the metabolic pool and are utilized for various purposes.



Sites of action of the phospholipases A_1, A_2, C and D.

The products of these phospholipases are called lysophospholipids and can be substrates for acyl transferases utilizing different acyl-CoA groups.

Cholesterol

Cholesterol is a waxy steroid of fat that is produced in the liver or intestines. It is used to produce hormones and cell membranes and is transported in the blood plasma of all mammals. It is an essential structural component of mammalian cell membranes and is required to establish proper membrane permeability and fluidity. In addition, cholesterol is an important component for the manufacture of bile acids, steroid hormones, and vitamin D. Cholesterol is the principal sterol synthesized by animals; however, small quantities can be synthesized in other eukaryotes such as plants and fungi. It is almost completely absent among prokaryotes including bacteria. Although cholesterol is important and necessary for mammals, high levels of cholesterol in the blood have been linked to damage to arteries and are potentially linked to diseases such as those associated with the cardiovascular system (heart disease).

Since cholesterol is essential for all animal life, it is primarily synthesized from simpler substances within the body. However, high levels in blood circulation, depending on how it is transported within lipoproteins, are strongly associated with progression of atherosclerosis. For a person of about 68 kg, typical total body cholesterol synthesis is about 1 g (1,000 mg) per day, and total body content is about 35 g.

Cholesterol is recycled. It is excreted by the liver via the bile into the digestive tract. Typically, about 50% of the excreted cholesterol is reabsorbed by the small bowel back into the bloodstream. Phytosterols can compete with cholesterol reabsorption in the intestinal tract, thus reducing cholesterol reabsorption.

Functions of cholesterol

- Cholesterol is required to build and maintain membranes; it modulates membrane fluidity over the range of physiological temperatures.

  The hydroxyl group on cholesterol interacts with the polar head groups of the membrane phospholipids and sphingolipids, while the bulky steroid and the hydrocarbon chain are embedded in the membrane, alongside the nonpolar fatty acid chain of the other lipids. Through the interaction with the phospholipid fatty acid chains, cholesterol increases membrane packing, which reduces membrane fluidity. In this structural role, cholesterol reduces the permeability of the plasma membrane to neutral solutes, protons, (positive hydrogen ions) and sodium ions.

- In many neurons, a myelin sheath, rich in cholesterol, provides insulation for more efficient conduction of impulses.

- Within cells, cholesterol is the precursor molecule in several biochemical pathways. In the liver, cholesterol is converted to bile, which is then stored in the gallbladder. Bile contains bile salts, which solubilize fats in the digestive tract and aid in the intestinal absorption of fat molecules as well as the fat-soluble vitamins, A, D, E, and K.
- Cholesterol is an important precursor molecule for the synthesis of vitamin D and the steroid hormones, including the adrenal gland hormones cortisol and aldosterone, as well as the sex hormones progesterone, estrogens, and testosterone, and their derivatives.

Biosynthesis of cholesterol

All animal cells manufacture cholesterol with relative production rates varying by cell type and organ function. About 20–25% of total daily cholesterol production occurs in the liver; other sites of higher synthesis rates include the intestines, adrenal glands, and reproductive organs.The enzymes involved in the synthesis of cholesterol are partly located in the endoplasmic reticulum and partly in the cytoplasm.

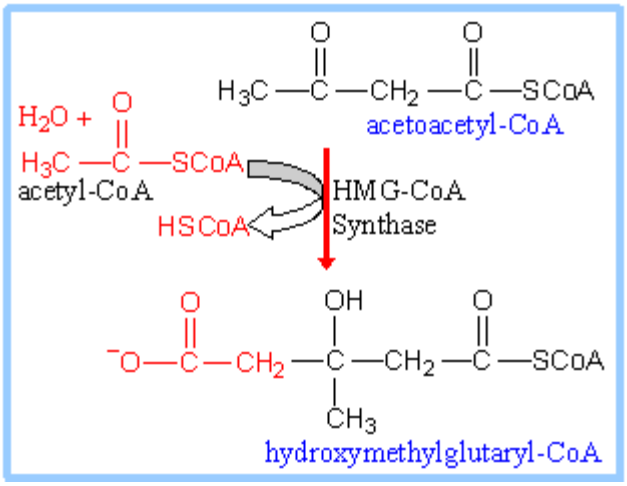
The starting material for cholesterol synthesis is acetyl CoA which is formed inside the mitochondria from pyruvate. The inner mitochondrial membrane is not freely permeable to acetyl CoA; hence the acetyl CoA is delivered to the cytoplasm as citrate which is transported by a tricarboxylic transporter. Here in the cytoplasm, the citrate is cleaved to oxaloacetate and acetyl CoA by the enzyme **ATP citrate lyase**. The oxaloacetate returns to the mitochondria as malate or pyruvate.

Step 1: condensation

Two molecules of acetyl CoA condense to form acetoacetyl CoA. The reaction is catalysed by the enzyme **acetoacetyl CoA synthase.**

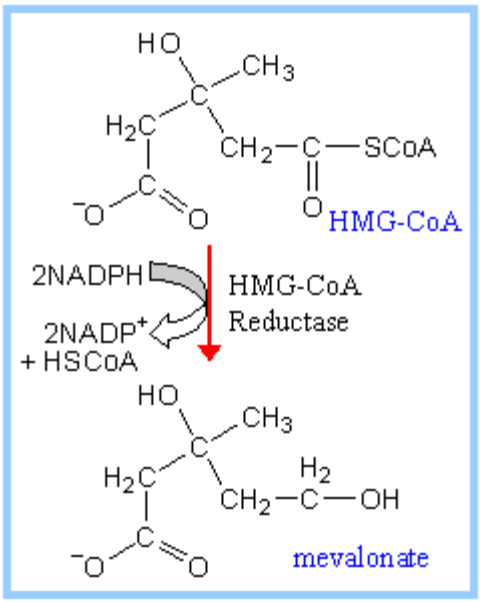
Step 2: Formation of Hydroxymethylglutaryl-coenzyme A (HMG-CoA)

HMG-CoA is formed by condensation of acetyl-CoA and acetoacetyl-CoA, catalyzed by **HMG-CoA Synthase**.



Step 3: The commited step

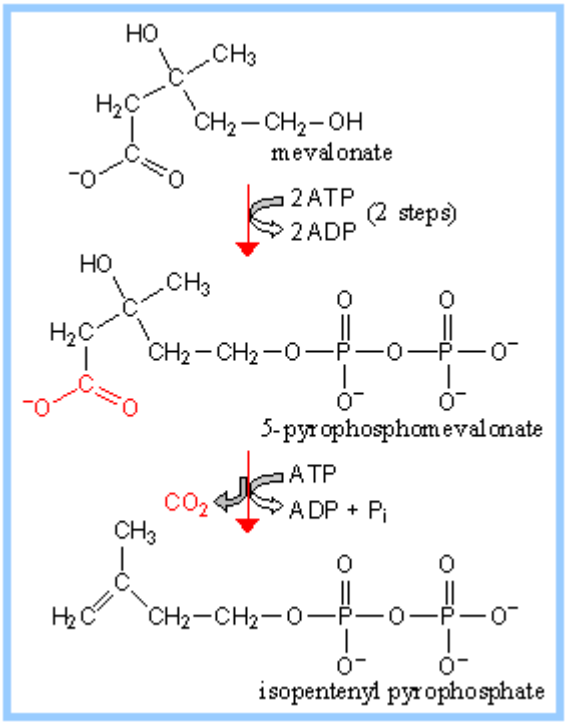
HMG-CoA Reductase catalyzes production of mevalonate from HMG-CoA. The carboxyl group of hydroxymethylglutarate that is in ester linkage to the thiol of coenzyme A is reduced first to an aldehyde and then to an alcohol. NADPH serves as reductant in the 2-step reaction. HMG-CoA Reductase is an integral protein of endoplasmic reticulum membranes.



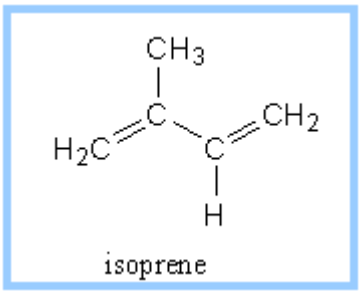
The HMG-CoA Reductase reaction is rate-limiting for cholesterol synthesis. This enzyme is highly regulated and the target of pharmaceutical intervention.

Step 4: Production of five carbon (isoprenoid) unit

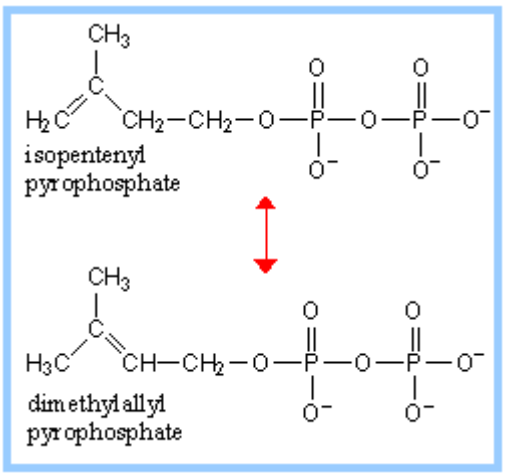
Mevalonate is phosphorylated by 2 sequential phosphate transfers from ATP, yielding the pyrophosphate derivative. Pyrophosphomevolanate decarboxylase catalyzes ATP-dependent decarboxylation, with dehydration, to yield isopentenyl pyrophosphate.



Isopentenyl pyrophosphate is the first of several compounds in the pathway that are referred to as isoprenoids, by reference to the compound isoprene.

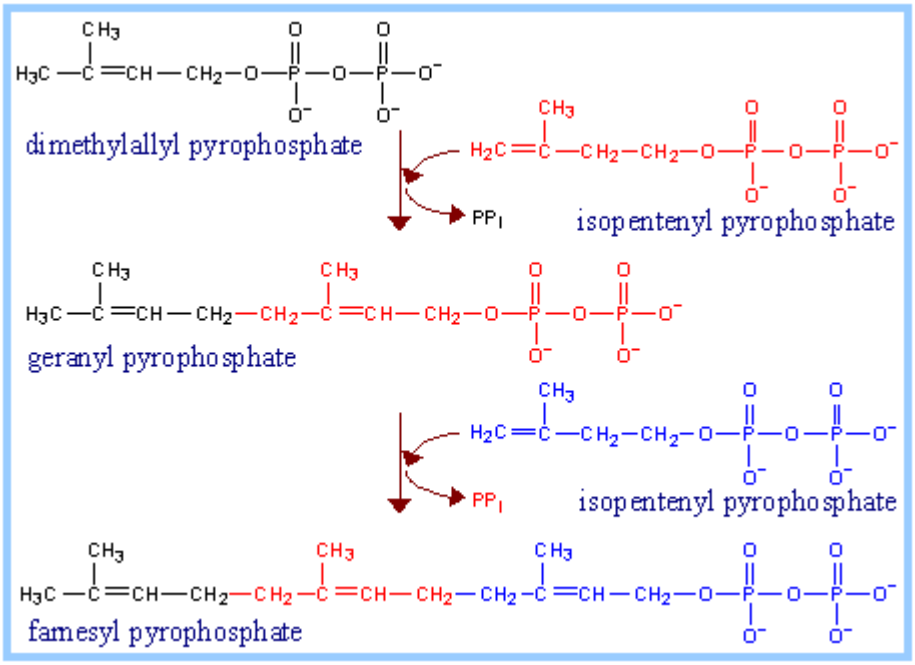


Isopentenyl Pyrophosphate Isomerase inter-converts isopentenyl pyrophosphate and dimethylallyl pyrophosphate.
The mechanism involves protonation followed by deprotonation.



Step 5: Condensation of five carbon units (synthesis of squalene)

Prenyl Transferase (farnesyl pyrophosphate synthase) catalyzes a series of head-to-tail condensation reactions. Dimethylallyl pyrophosphate reacts with isopentenyl pyrophosphate to form geranyl pyrophosphate. Condensation with another isopentenyl pyrophosphate yields farnesyl pyrophosphate.

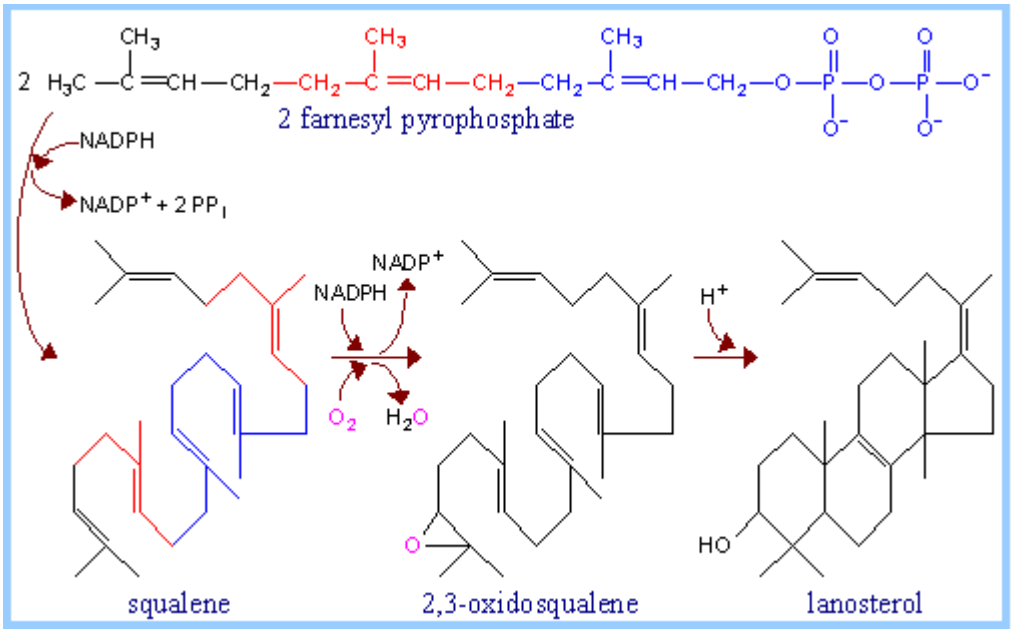


Squalene Synthase catalyzes head-to-head condensation of 2 molecules of farnesyl pyrophosphate, with reduction by NADPH, to yield squalene.

Step 6: Cyclization

Squalene epoxidase catalyzes oxidation of squalene to form 2, 3-oxidosqualene. This mixed function oxidation requires NADPH as reductant and O_2 as oxidant. One atom of oxygen is incorporated into the substrate (as the epoxide) and the other oxygen atom is reduced to water.

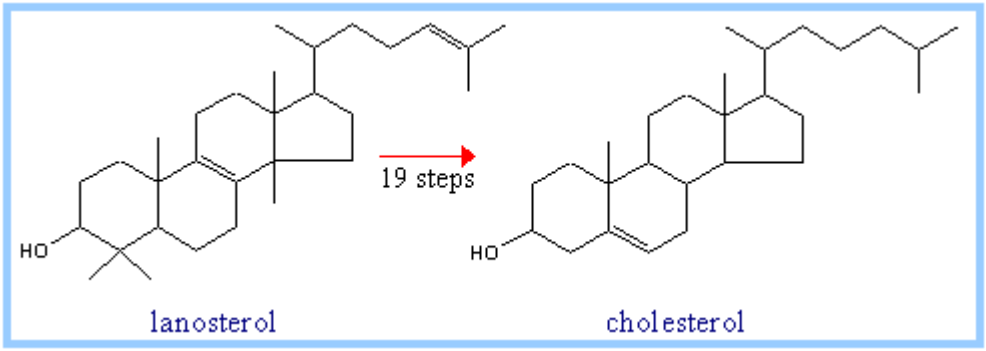
Squalene Oxidocyclase catalyzes a series of electron shifts, initiated by protonation of the epoxide, resulting in cyclization. The product of the cyclization reaction is the sterol lanosterol.



Step 7: cutting to size

Conversion of lanosterol to cholesterol involves 19 reactions, catalyzed by enzymes associated with endoplasmic reticulum membranes. The 3 additional methy groups on carbon atoms 4 and 14 are removed to produce zymosterol.

Then the double bond migrates from 8-9 position to 5-6 position, when desmosterol is formed. Finally, the double bond in the side chain between carbons 24-25 is reduced by NADPH when cholesterol is formed.



Additional modifications yield the various steroid hormones or vitamin D. Many of the reactions involved in converting lanosterol to cholesterol and other steroids are catalyzed by members of the cytochrome P_{450} enzyme superfamily.

Regulation of cholesterol synthesis

HMG-CoA Reductase, the rate-determining step on the pathway for synthesis of cholesterol, is a major control point.

Short-term regulation

- **Covalent modification**

HMG-CoA Reductase is inhibited by phosphorylation, catalyzed by AMP-Dependent Protein Kinase (which also regulates fatty acid synthesis and catabolism). This kinase is active when cellular AMP is high, corresponding to when ATP is low. Thus, when cellular ATP is low, energy is not expended in synthesizing cholesterol.

Long-term regulation of cholesterol synthesis is by varied formation and degradation of HMG-CoA Reductase and other enzymes of the pathway for synthesis of cholesterol.

- **Regulated proteolysis of HMG-CoA Reductase:** Degradation of HMG-CoA Reductase is stimulated by cholesterol, by oxidized derivatives of cholesterol, by mevalonate, and by farnesol (dephosphorylated farnesyl pyrophosphate). HMG-CoA Reductase includes a transmembrane sterol-sensing domain that has a role in activating degradation of the enzyme via the proteasome.

- **Regulated transcription:** A family of transcription factors designated SREBP (sterol regulatory element binding proteins) regulate synthesis of cholesterol and fatty acids. Of these, SREBP-2 mainly regulates cholesterol synthesis.
  When sterol levels are low, SREBP-2 is released by cleavage of a membrane-bound precursor protein. SREBP-2 activates transcription of genes for HMG-CoA Reductase and other enzymes of the pathway for cholesterol synthesis. But when sufficient cholesterol is present in the cell, transcription of the gene for HMG CoA reductase is suppressed, and cellular synthesis of cholesterol is decreased.
- **Hormonal regulation:**

Insulin and thyroxine increase the activity of HMG CoA reductase by enhancing the formation of active HMG-CoA reductase (dephosphorylated form); whereas cortisol and glucagon decreases its activity by favouring the formation of the inactive HMG-CoA reductase (phosphorylated form).

- **Inhibition by drugs**

  Drugs used to inhibit cholesterol synthesis include competitive inhibitors of HMG-CoA Reductase. Examples include various statin drugs such as lovastatin (Mevacor) and derivatives (e.g., Zocor), Lipitor, etc. A portion of each statin is analogous in structure to mevalonate or to the postulated mevaldehyde intermediate. Extensive clinical trials have shown that the statin drugs decrease blood cholesterol and diminish risk of cardiovascular disease.

- HMG-CoA reductase activity is also inhibited by bile acids. Fasting also reduces the activity of this enzyme.

  Degradation of cholesterol

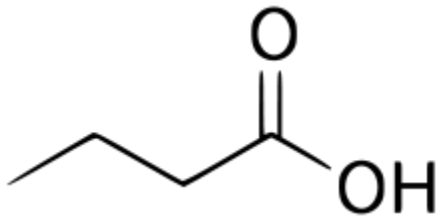
  Cholesterol is susceptible to oxidation and easily forms oxygenated derivatives known as oxysterols. A great interest in oxysterols arose when they were shown to exert inhibitory actions on cholesterol biosynthesis. Additional roles for oxysterols in human physiology include their: participation in bile acid biosynthesis, function as transport forms of cholesterol, and regulation of gene transcription.

  Cholesterol is oxidized by the liver into a variety of bile acids. These, in turn, are conjugated with glycine, taurine, glucuronic acid, or sulfate. A mixture of conjugated and nonconjugated bile acids, along with cholesterol itself, is excreted from the liver into the bile. Approximately 95% of the bile acids are reabsorbed from the intestines, and the remainder is lost in the feces.

  Under certain circumstances, when more concentrated, as in the gallbladder, cholesterol crystallises and is the major constituent of most gallstones.

  Every day, up to 1 g of cholesterol enters the colon. This cholesterol originates from the diet, bile, and desquamated intestinal cells, and can be metabolized by the colonic bacteria. Cholesterol is mainly converted into coprostanol, a nonabsorbable sterol which is excreted in the feces.

Fatty acids



Butyric acid, a short-chain fatty acid

Fatty acid is a carboxylic acid with a long unbranched aliphatic tail (chain), which is either saturated or unsaturated. They can also be defined as aliphatic monocarboxylic acids derived from, or contained in esterified form in, an animal or vegetable fat, oil, or wax. Most naturally occurring fatty acids have a chain of four to 28 carbons. The number of carbon atoms is usually even, because their biosynthesis involves acetyl-CoA, a coenzyme carrying a two-carbon-atom group.

It is proposed that the blends of fatty acids exuded by mammalian skin, together with lactic acid and pyruvic acid, are distinctive and enable animals with a keen sense of smell to differentiate individuals.

Types

Fatty acids can be saturated and unsaturated, depending on double bonds. They differ in length as well.

Saturated fatty acids

Saturated fatty acids are long-chain carboxylic acids that usually have between 12 and 24 carbon atoms and have no double bonds. Thus, saturated fatty acids are saturated with hydrogen (since double bonds reduce the number of hydrogens on each carbon). Because saturated fatty acids have only single bonds, each carbon atom within the chain has 2 hydrogen atoms (except for the omega carbon at the end that has 3 hydrogens).

Example; (1) Lauric acid (12 C), (2) Myristic acid (14 C), (3) Palmitic acid (16 C), (4) Stearic acid (18 C), (5) Arachidic acid (20 C).

Unsaturated fatty acids

Unsaturated fatty acids resemble saturated fatty acids, except that the chain has one or more double-bonds. Fatty acids with one double bond are monounsaturated, and those with 2 or more double bonds are collectively known as polyunsaturated fatty acids (PUFA).

Examples of unsaturated fatty acids:

Common name	Chemical structure
Myristoleic acid	$CH_3(CH_2)_3\mathbf{CH=CH}(CH_2)_7COOH$
Palmitoleic acid	$CH_3(CH_2)_5\mathbf{CH=CH}(CH_2)_7COOH$
Sapienic acid	$CH_3(CH_2)_8\mathbf{CH=CH}(CH_2)_4COOH$
Oleic acid	$CH_3(CH_2)_7\mathbf{CH=CH}(CH_2)_7COOH$
Linoleic acid	$CH_3(CH_2)_4\mathbf{CH=CH}CH_2\mathbf{CH=CH}(CH_2)_7COOH$
α-Linolenic acid	$CH_3CH_2\mathbf{CH=CH}CH_2\mathbf{CH=CH}CH_2\mathbf{CH=CH}(CH_2)_7COOH$

Arachidonic acid $CH_3(CH_2)_4\mathbf{CH=CH}CH_2\mathbf{CH=CH}CH_2\mathbf{CH=CH}CH_2\mathbf{CH=CH}(CH_2)_3COOH$

Essential fatty acids

Fatty acids that are required by the body, but cannot be made in sufficient quantity by the body from other substrates, therefore must be obtained from food and are called essential fatty acids. In the body, essential fatty acids are primarily used to produce hormone-like substances that regulate a wide range of functions, including blood pressure, blood clotting, blood lipid levels, the immune response, and the inflammation response to injury infection.

The human body can produce all but two of the fatty acids it needs. These two, linoleic acid (LA) and alpha-linolenic acid (ALA) are widely distributed in plant oils.

They are essential in the human diet because, like all mammals, they lack the ability to introduce double bonds in fatty acids beyond carbons 9 and 10, as counted from the carboxylic acid side, because they do not have the enzymes necessary to introduce a double bond at the omega-3 position or omega-6 position. Humans can easily make saturated fatty acids or monounsaturated fatty acids with a double bond at the omega-9 position.

Long and short chain fatty acids

In addition to saturation, fatty acids are short, medium, or long.

- Short-chain fatty acids (SCFA) are fatty acids with aliphatic tails of fewer than six carbons.
- Medium-chain fatty acids (MCFA) are fatty acids with aliphatic tails of 6–12 carbons, which can form medium-chain triglycerides.
- Long-chain fatty acids (LCFA) are fatty acids with aliphatic tails longer than 12 carbons
- Very-Long-chain fatty acids (VLCFA) are fatty acids with aliphatic tails longer than 22 carbons

Free fatty acids

Fatty acids can be bound or attached to other molecules, such as in triglycerides or phospholipids. When they are not attached to other molecules, they are known as "free" fatty acids.

The uncombined fatty acids or free fatty acids may come from the breakdown of a triglyceride into its components (fatty acids and glycerol). However, as fats are insoluble in water, they must be bound to appropriate regions in the plasma protein albumin for transport around the body. The levels of "free fatty acid" in the blood are limited by the number of albumin binding sites available.

Free fatty acids are an important source of fuel for many tissues since they can yield relatively large quantities of ATP. Many cell types can use either glucose or fatty acids

for this purpose. In particular, heart and skeletal muscle prefer fatty acids. The brain cannot use fatty acids as a source of fuel; it relies on glucose, or on ketone bodies.

Synthesis of fatty acids

Fatty acids are mainly synthesized by a *de novo* pathway operating in the cytoplasm. So it is refered to as extramitochondrial or cytoplasmic fatty acid synthase system. The major fatty acid synthesized is palmitic acid, the 16C saturated fatty acid. Fatty acid synthesis occurs in the liver, adipose tissue, kidney, brain and mammary glands.

Fatty acid synthase (FAS) complex:

This is a multi-enzyme complex which forms a dimer with identical subunits. Each of the subunits is organized into 3 dormains.

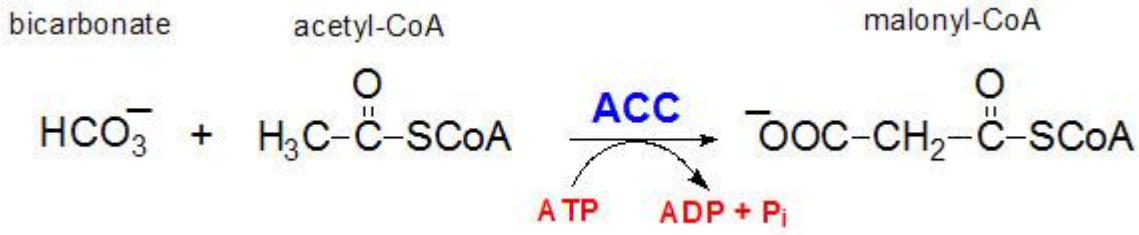
1st domain: This is also known as the condensing unit. It provides the initial substrate binding site. The enzymes involved are the condensing enzyme (CE) also known as the beta-keto acyl synthase, acety transferase (AT), and the malonyl trans acylase (MT).

2nd domain: This is the reduction unit. It contains the enzymes dehydratase (DH), enoyl reductase (ER), beta-keto acyl reductase (KR) and acyl carrier protein (ACP). ACP acts like CoA carrying fatty acyl groups. The acyl groups are attached in a thioester linkage.

3rd domain: This is the releasing unit. It is involved in the release of the fatty acid synthesized. It contains the enzyme thio-esterase (TE), which is also known as a de-acylase enzyme.

Step 1: Carboxylation of acetyl CoA

The synthesis of malonyl-CoA is the first committed step of fatty acid synthesis and the enzyme that catalyzes this reaction, acetyl-CoA carboxylase (ACC), is the major site of regulation of fatty acid synthesis. Like other enzymes that transfer CO_2 to substrates, ACC requires a biotin co-factor.



Step two: Three carbon and two carbon units are added.

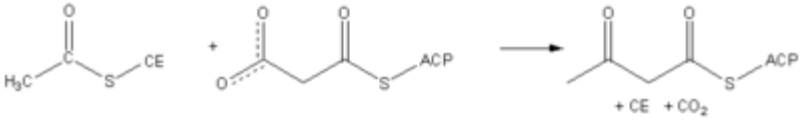
The acetyl-CoA and malonyl-CoA are transferred to ACP by the action of acetyl-CoA transacylase and malonyl-CoA transacylase, respectively. The attachment of these carbon atoms to ACP allows them to enter the fatty acid synthesis cycle.

$CH_3 – CO - SCoA + (CE) – SH \rightarrow CH_3 – CO - S – (CE) + CoA$

$^-OOC\text{-}CH_2\text{-}CO\text{-}SCoA + ACP – SH \rightarrow {}^-OOC\text{-}CH_2\text{-}CO – S – ACP + CoA$

Step 3: Condensation

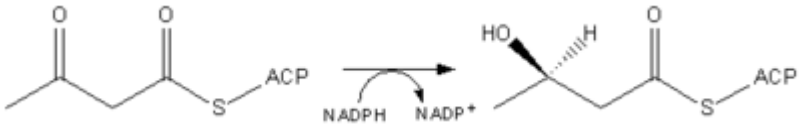
The condensation of acetyl ACP and malonyl ACP results in the formation of acetoacetyl ACP or beta- keto acyl ACP (4C). During this process, one carbon is lost as CO_2. The enzyme is beta- keto acyl synthase or CE.



Although this reaction is thermodynamically unfavourable, the evolution of CO_2 drives the reaction forward.

Step 4: Reduction of acetoacetyl ACP

The acetoacetyl ACP is reduced by NADPH dependent beta- keto acyl reductase to form beta –hydroxy butyryl ACP. The double bond is reduced to a hydroxyl group.



Acetoacetyl ACP Beta –hydroxy butyryl ACP.

Step 5: Dehydration of β-Hydroxybutyryl ACP

β-Hydroxybutyryl ACP is then dehydrated by β-hydroxyacyl-ACP dehydratase to form enoyl ACP otherwise known as alpha beta unsaturated acyl ACP. In this step, Water is drawn out creating a double bond between the β and Gamma carbons.

$ACP–S –CO\text{-} CH_2 –CHOH –CH_3$----------->$ACP–S–CO –CH =CH\text{-} CH_3$ (enoyl ACP)

Step 6: Second reduction

The enoyl ACP is again reduced by enoyl ACP reductase, utilizing a 2nd molecule of NADPH to form butyryl ACP.

(Butyryl ACP).

Cycling of reactions

Butyryl CoA is then translocated in the CE site, and another malonyl CoA is brought in the ACP site. In the second step of elongation, butyryl-CE condenses with malonyl ACP to form an acyl ACP compound. This continues until a C_{16} acyl compound is formed, at which point it is hydrolyzed by a thioesterase into palmitate and ACP.

The end-product of these reactions is always palmitate. No intermediates are released until palmitate is formed. After release from the ACP, palmitate is esterified to Coenzyme A, as this is done with all free fatty acids inside cells to prevent lysis of the cell membranes. If further elongation has to happen, the palmitoyl CoA, or any other acyl-CoA from a dietary acid, then moves into the endoplasmic reticulum, where it can be elongated up to a length of 20 to 24 carbons by the same chain of reactions as happens in the cytosolic ACP.

Summary of de novo synthesis of fatty acid

The net reaction is as shown:

$$1\text{Acetyl CoA} + 7\text{malonyl CoA} + 14\text{NADPH} + 14H^+ \rightarrow 1\text{palmitate} + 7CO_2 + 14NADP^+ + 8\text{CoA} + 6H_2O.$$

Regulation of fatty acid synthesis

Acetyl CoA carboxylase (ACC) are allosterically activated by citrate and inhibited by palmitoyl-CoA and other short- and long-chain fatty acyl-CoAs. Citrate level is high only when both acetyl CoA and ATP are abundant.

ACC activity can also be affected by phosphorylation. Phosphorylation of ACC at three serine residues (S79, S1200, and S1215) by AMPK leads to inhibition of the enzyme.

The activating effects of insulin on ACC are complex and not completely resolved. It is known that insulin leads to the dephosphorylation of the serines in ACC. This insulin-mediated effect has not been observed in hepatocytes or adipose tissues cells.

Control of a given pathways' regulatory enzymes can also occur by alteration of enzyme synthesis and turn-over rates. These changes are long term regulatory effects. Insulin stimulates ACC and FAS synthesis, whereas, starvation leads to decreased synthesis of these enzymes.

Glucagon and epinephrine inactivates the acetyl CoA carboxylase enzyme by phosphorylating the enzyme.

Fatty acid synthesis occurs when carbohydrate is abundant and the level of fatty acids is low. The availability of citrate in the cytoplasm is the most important regulatory factor for producing a short term effect.

Beta Oxidation of fatty acids

Introduction

Utilization of dietary lipids requires that they first be absorbed through the intestine. As these molecules are oils they would be essentially insoluble in the aqueous intestinal environment. Solubilization (emulsification) of dietary lipid is accomplished via bile salts that are synthesized in the liver and secreted from the gallbladder.

The emulsified fats can then be degraded by pancreatic lipases (lipase and phospholipase A_2). These enzymes, secreted into the intestine from the pancreas, generate free fatty acids and a mixture of mono- and diacylglycerols from dietary triacylglycerols. Pancreatic lipase degrades triacylglycerols at the 1 and 3 positions sequentially to generate 1, 2-diacylglycerols and 2-acylglycerols. Phospholipids are degraded at the 2 position by pancreatic phospholipase A_2 releasing a free fatty acid and the lysophospholipid.

Following absorption of the products of pancreatic lipase by the intestinal mucosal cells, the resynthesis of triacylglycerols occurs. The triacylglycerols are then solubilized in lipoprotein complexes (complexes of lipid and protein) called **chylomicrons**. Triacylglycerols synthesized in the liver are packaged into VLDLs and released into the blood directly. Chylomicrons from the intestine are then released into the blood via the lymph system for delivery to the various tissues for storage or production of energy through oxidation.

The triacylglycerol components of VLDLs and chylomicrons are hydrolyzed to free fatty acids and glycerol in the capillaries of adipose tissue and skeletal muscle by the action of lipoprotein lipase. The free fatty acids are then absorbed by the cells and the glycerol is returned via the blood to the liver (and kidneys). The glycerol is then converted to the glycolytic intermediate DHAP.

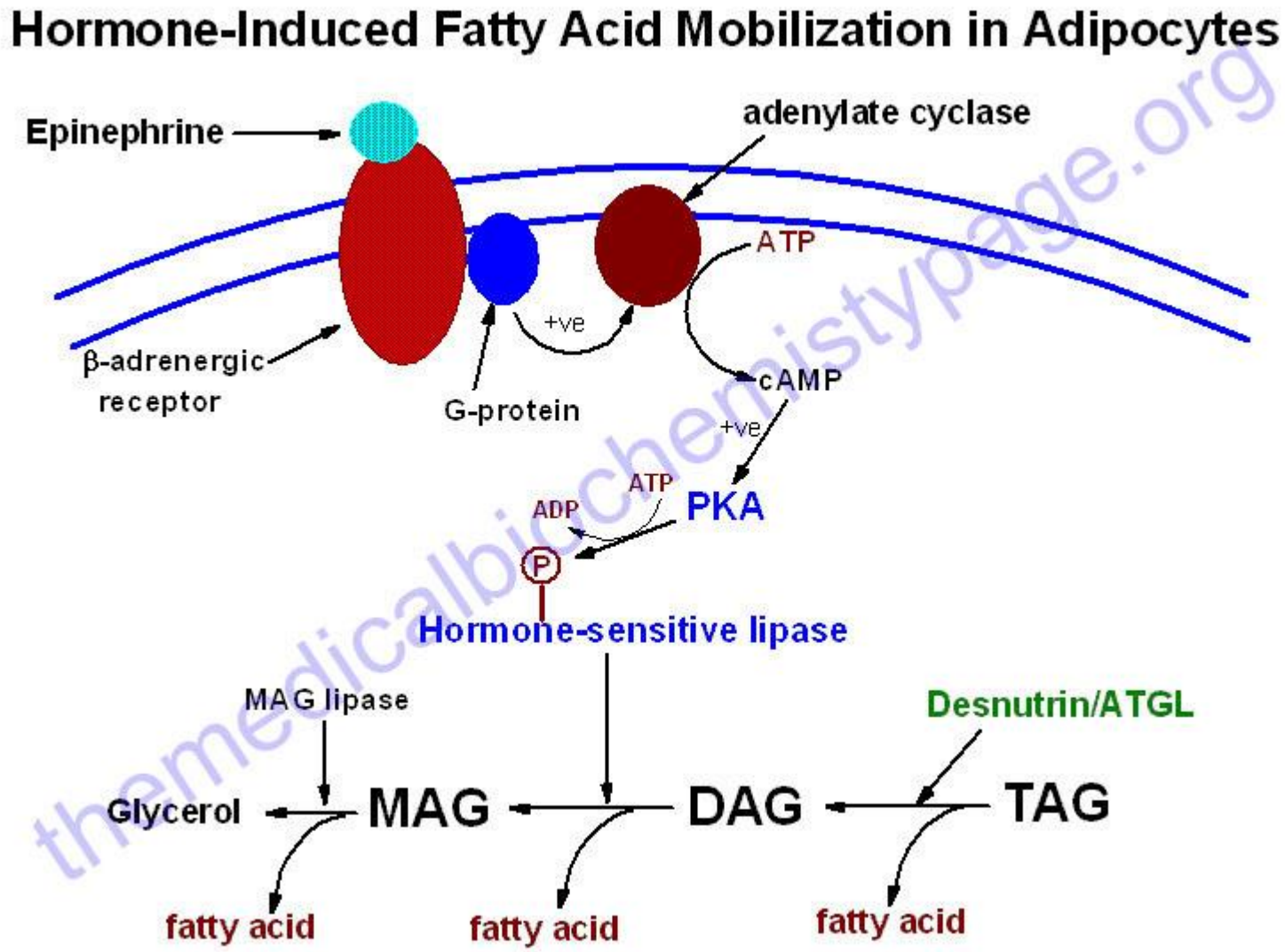
Mobilization of Fat Stores

The primary sources of fatty acids for oxidation are dietary and mobilization from cellular stores. Fatty acids from the diet can are delivered from the gut to cells via transport in the blood. Fatty acids are stored in the form of triacylglycerols primarily within adipocytes of adipose tissue. In response to energy demands, the fatty acids of stored triacylglycerols can be mobilized for use by peripheral tissues. The release of metabolic energy, in the form of fatty acids, is controlled by a complex series of interrelated cascades that result in the activation of hormone-sensitive lipase.

The stimulus to activate this cascade, in adipocytes, can be glucagon, epinephrine or β-corticotropin. These hormones bind cell-surface receptors that are coupled to the activation of adenylate cyclase upon ligand binding. The resultant increase in cAMP leads to activation of PKA, which in turn phosphorylates and activates hormone-sensitive lipase (HSL).

This enzyme hydrolyzes fatty acids from carbon atoms 1 or 3 of diacylglycerols. The diacylglycerols are the products of the action of the triacylglycerol lipase identified as desnutrin (also called adipose tissue triacylglycerol lipase, ATGL). Desnutrin/ATGL is specific for triacylglycerols and provides the diacylglycerols acted upon when HSL is activated. The

monacylglycerols that result from the action of HSL are substrates for monoacylglycerol lipase. The net result of the action of these enzymes is three moles of free fatty acid and one mole of glycerol. The free fatty acids diffuse from adipose cells, combine with albumin in the blood, and are thereby transported to other tissues, where they passively diffuse into cells.



Model for the activation of hormone-sensitive lipase by epinephrine. Epinephrine binds its receptor and leads to the activation of adenylate cyclase. The resultant increase in cAMP activates PKA which then phosphorylates and activates hormone-sensitive lipase. Hormone-sensitive lipase hydrolyzes fatty acids from diacylglycerols that result from the action of the hormone-insensitive lipase, desnutrin/ATGL. The final fatty acid is released from monoacylglycerols through the action of monoacylglycerol lipase, an enzyme that is also active in the absence of hormonal stimulation.

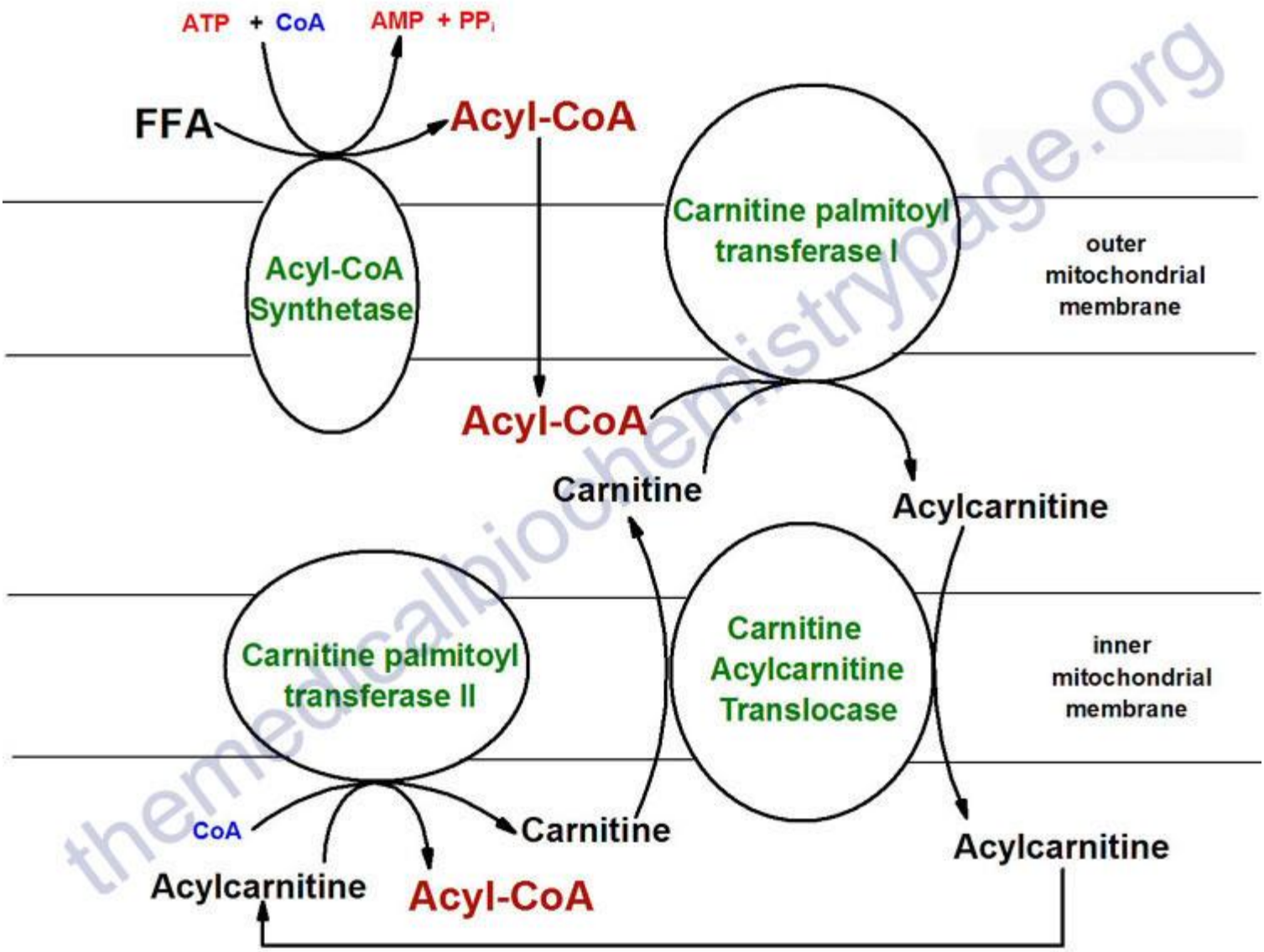
In contrast to the hormonal activation of adenylate cyclase and (subsequently) hormone-sensitive lipase in adipocytes, the mobilization of fat from adipose tissue is inhibited by numerous stimuli. The most significant inhibition is that exerted upon adenylate cyclase by insulin. When an individual is well fed state, insulin released from the pancreas prevents the inappropriate mobilization of stored fat. Instead, any excess fat and carbohydrate are incorporated into the triacylglycerol pool within adipose tissue.

Reactions of Oxidation

Fatty acids must be activated in the cytoplasm before being oxidized in the mitochondria. Activation is catalyzed by fatty acyl-CoA ligase (also called acyl-CoA synthetase or thiokinase). The net result of this activation process is the consumption of 2 molar equivalents of ATP.

Fatty acid + ATP + CoA ——> Acyl-CoA + PP_i + AMP

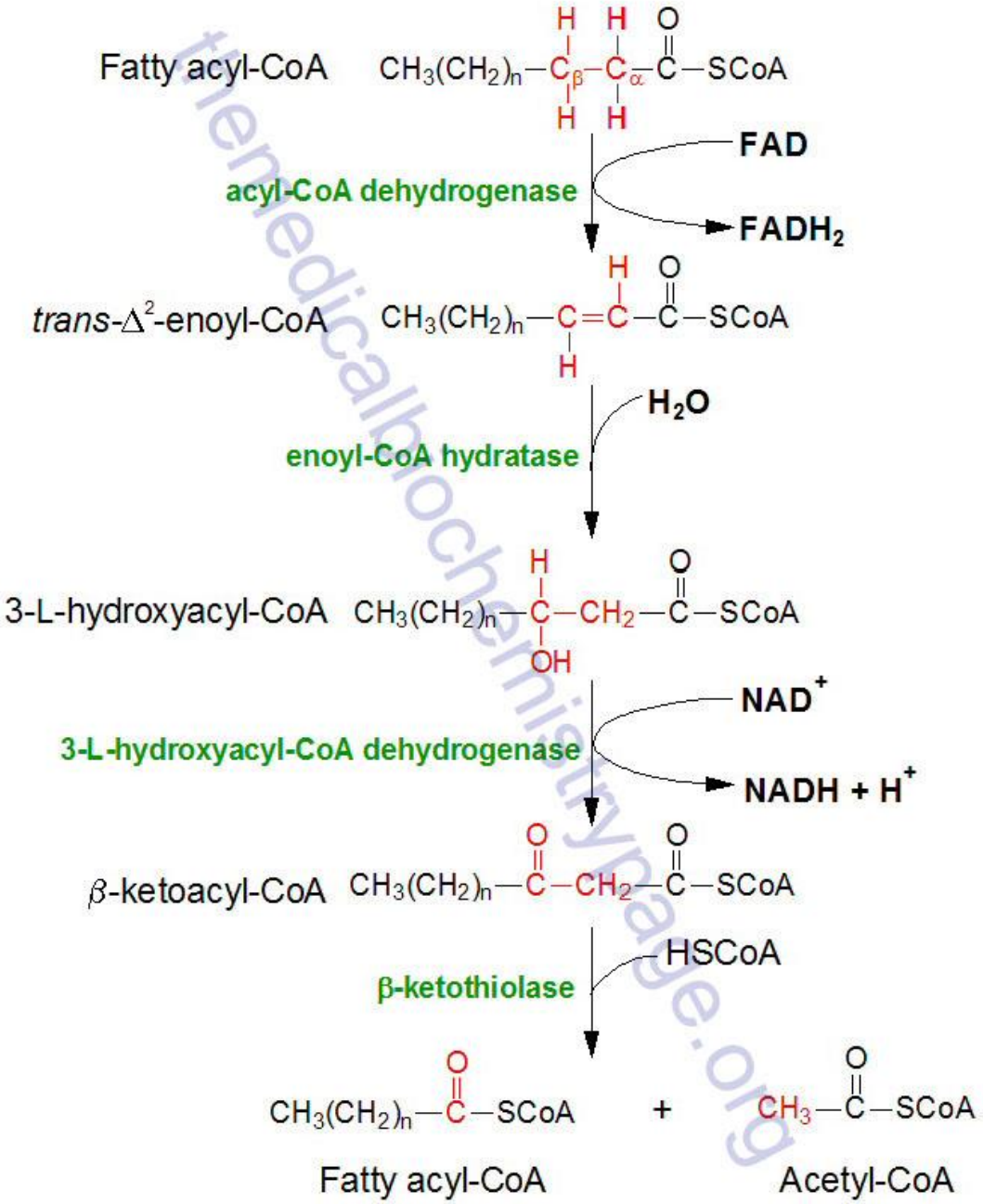
Oxidation of fatty acids occurs in the mitochondria. The transport of fatty acyl-CoA into the mitochondria is accomplished via an acyl-carnitine intermediate, which itself is generated by the action of carnitine palmitoyltransferase I (CPT I, also called carnitine acyltransferase I, CA I) an enzyme that resides in the outer mitochondrial membrane. The acyl-carnitine molecule then is transported into the mitochondria where carnitine palmitoyltransferase II (CPT II, also called carnitine acyltransferase II, CA II) catalyzes the regeneration of the fatty acyl-CoA molecule.



Transport of fatty acids from the cytoplasm to the inner mitochondrial space for oxidation. Following activation to a fatty-CoA, the CoA is exchanged for carnitine by CPT I. The fatty-carnitine is then transported to the inside of the mitochondrion where a reversal exchange takes place through the action of CPT II. Once inside the mitochondrion the fatty-CoA is a substrate for the β-oxidation machinery.

The process of fatty acid oxidation is termed β-oxidation since it occurs through the sequential removal of 2-carbon units by oxidation at the β-carbon position of the fatty acyl-CoA molecule.

Each round of β-oxidation produces one mole of NADH, one mole of $FADH_2$ and one mole of acetyl-CoA. The acetyl-CoA, the end product of each round of β-oxidation, then enters the TCA cycle, where it is further oxidized to CO_2 with the concomitant generation of three moles of NADH, one mole of $FADH_2$ and one mole of ATP. The NADH and $FADH_2$ generated during the fat oxidation and acetyl-CoA oxidation in the TCA cycle then can enter the respiratory pathway for the production of ATP.

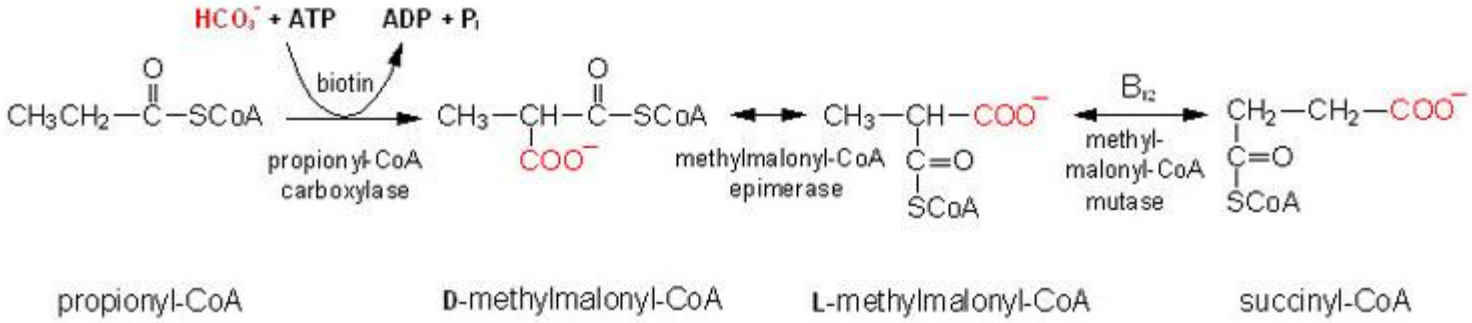


Pathway of β-Oxidation

The oxidation of fatty acids yields significantly more energy per carbon atom than does the oxidation of carbohydrates. The net result of the oxidation of one mole of oleic acid (an 18-carbon fatty acid) will be 146 moles of ATP (2 mole equivalents are used during the activation of the fatty acid), as compared with 114 moles from an equivalent number of glucose carbon atoms.

Alternative Oxidation Pathways

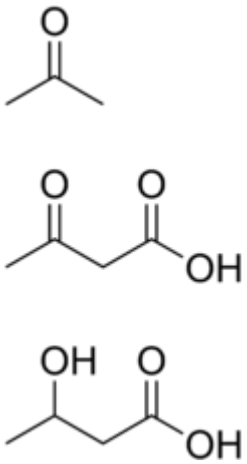
The majority of natural lipids contain an even number of carbon atoms. A small proportion of plant derived lipids contain odd numbers and upon complete β-oxidation these yield acetyl-CoA units plus a single mole of propionyl-CoA. The propionyl-CoA is converted, in an ATP-dependent pathway, to succinyl-CoA. The succinyl-CoA can then enter the TCA cycle for further oxidation.



Conversion of Propionyl-CoA to Succinyl-CoA

The oxidation of unsaturated fatty acids is essentially the same process as for saturated fats, except when a double bond is encountered. In such a case, the bond is isomerized by a specific enoyl-CoA isomerase and oxidation continues.

Ketone bodies



Chemical structures of the three ketone bodies: acetone (top), acetoacetic acid (middle), and beta-hydroxybutyric acid (bottom).

Ketone bodies are three water-soluble compounds that are produced as by-products when fatty acids are broken down for energy in the liver and kidney. They are used as a source of energy in the heart and brain. In the brain, they are a vital source of energy during fasting. Although termed "bodies", they are actually dissolved substances, not particles.

The three endogenous ketone bodies are acetone, acetoacetic acid, and beta-hydroxybutyric acid, although beta-hydroxybutyric acid is not technically a ketone but a carboxylic acid.

Uses in the heart and brain

Ketone bodies can be used for energy. They are transported from the liver to other tissues, where acetoacetate and beta-hydroxybutyrate can be reconverted to acetyl-CoA to produce energy, via the citric acid cycle. The heart gets little energy from ketone bodies except under special circumstances; it uses mainly fatty acids.

The brain gets a portion of its energy from ketone bodies when glucose is less available (e.g., during fasting, strenuous exercise, low carbohydrate, ketogenic diet and in neonates). In the event of low blood glucose, most other tissues have additional energy sources besides ketone bodies (such as fatty acids), but the brain does not. After the diet has been changed to lower blood glucose for 3 days, the brain gets 25% of its energy from ketone bodies. After about 4 days, this goes up to 70% (during the initial stages the brain does not burn ketones, because they are an important substrate for lipid synthesis in the brain).

Production of ketone bodies

Ketone bodies are produced from acetyl-CoA mainly in the mitochondrial matrix of hepatocytes when carbohydrates are so scarce that energy must be obtained from breaking down fatty acids. Because of the high level of acetyl CoA present in the cell, the pyruvate dehydrogenase complex is inhibited, whereas pyruvate carboxylase becomes activated. Thus, the oxaloacetate produced will enter gluconeogenesis rather than the citric acid cycle, as the latter is also inhibited by the elevated level of NADH resulting from ß-oxidation of fatty acids. The excess acetyl-CoA is therefore rerouted to ketogenesis. Such a state in humans is referred to as the fasted state.

Acetone is produced by spontaneous decarboxylation of acetoacetate, yielding levels of acetone much lower than those of other ketone bodies. Acetone cannot be converted back to acetyl-CoA, so it is excreted in the urine, or (as a consequence of its high vapor pressure) exhaled. Acetone is responsible for the characteristic "fruity" odor of the breath of persons in ketoacidosis.

Ketosis and ketoacidosis

Production of ketone bodies is known as ketogenesis, and this is necessary in small amounts. However, when excess ketone bodies accumulate, this abnormal state is called ketosis. Ketosis can be quantified by sampling the patient's exhaled air, and testing for acetone by gas chromatography. When even larger amounts of ketone bodies accumulate such that the blood's pH is lowered to dangerously acidic levels, this state is called ketoacidosis

Impact upon pH

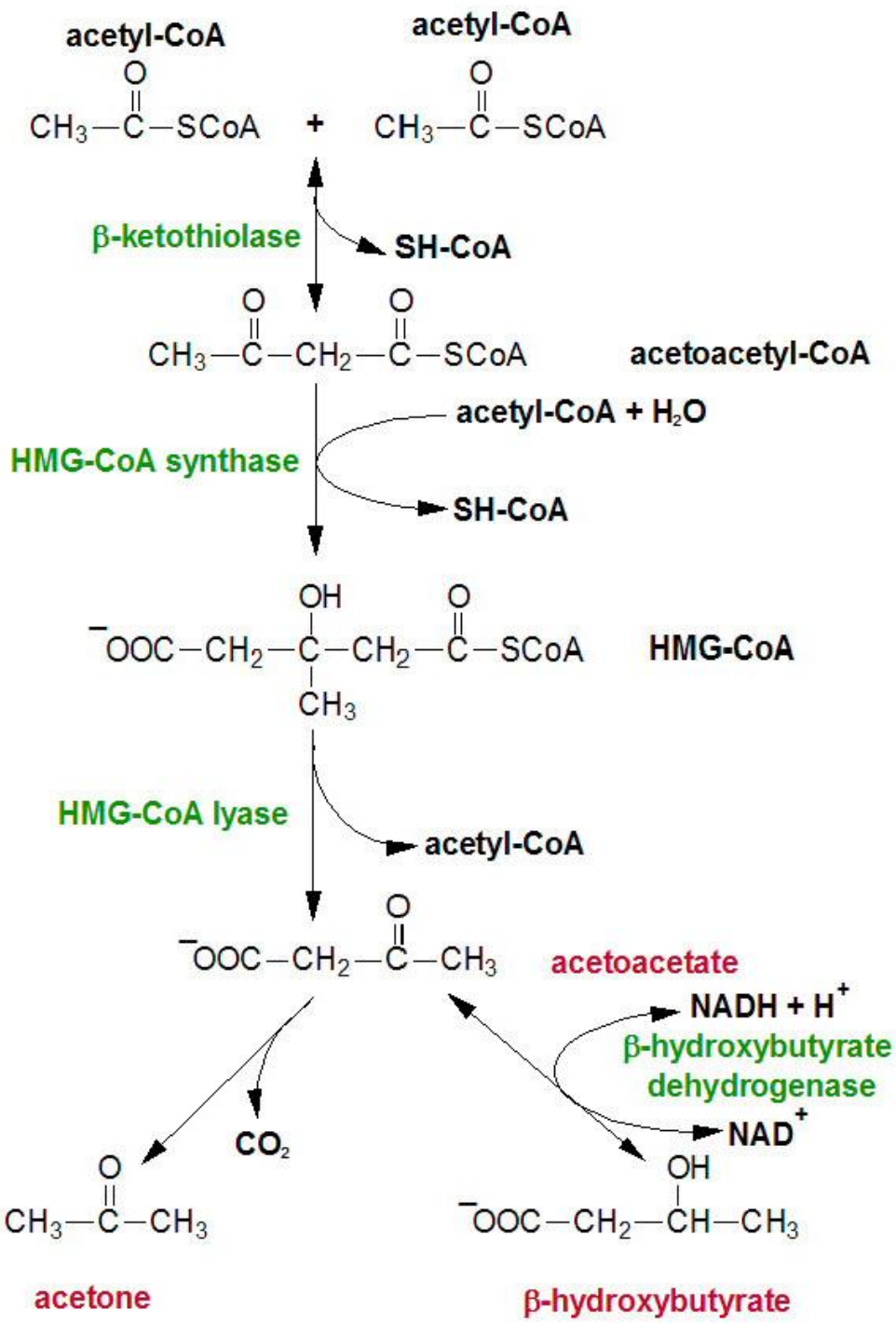
Both acetoacetic acid and beta-hydroxybutyric acid are acidic, and, if levels of these ketone bodies are too high, the pH of the blood drops, resulting in ketoacidosis.

This happens most often in untreated Type I diabetes, and somewhat less often in Type II.

Ketogenesis

During high rates of fatty acid oxidation, primarily in the liver, large amounts of acetyl-CoA are generated. These exceed the capacity of the TCA cycle, and one result is the synthesis of ketone bodies, or ketogenesis. The ketone bodies are **acetoacetate**, **β-hydroxybutyrate**, and **acetone**.

The formation of acetoacetyl-CoA occurs by condensation of two moles of acetyl-CoA through a reversal of the thiolase catalyzed reaction of fat oxidation. Acetoacetyl-CoA and an additional acetyl-CoA are converted to β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) by HMG-CoA synthase, an enzyme found in large amounts only in the liver. HMG-CoA in the mitochondria is converted to acetoacetate by the action of HMG-CoA lyase. Acetoacetate can undergo spontaneous decarboxylation to acetone, or be enzymatically converted to β-hydroxybutyrate through the action of β-hydroxybutyrate dehydrogenase.

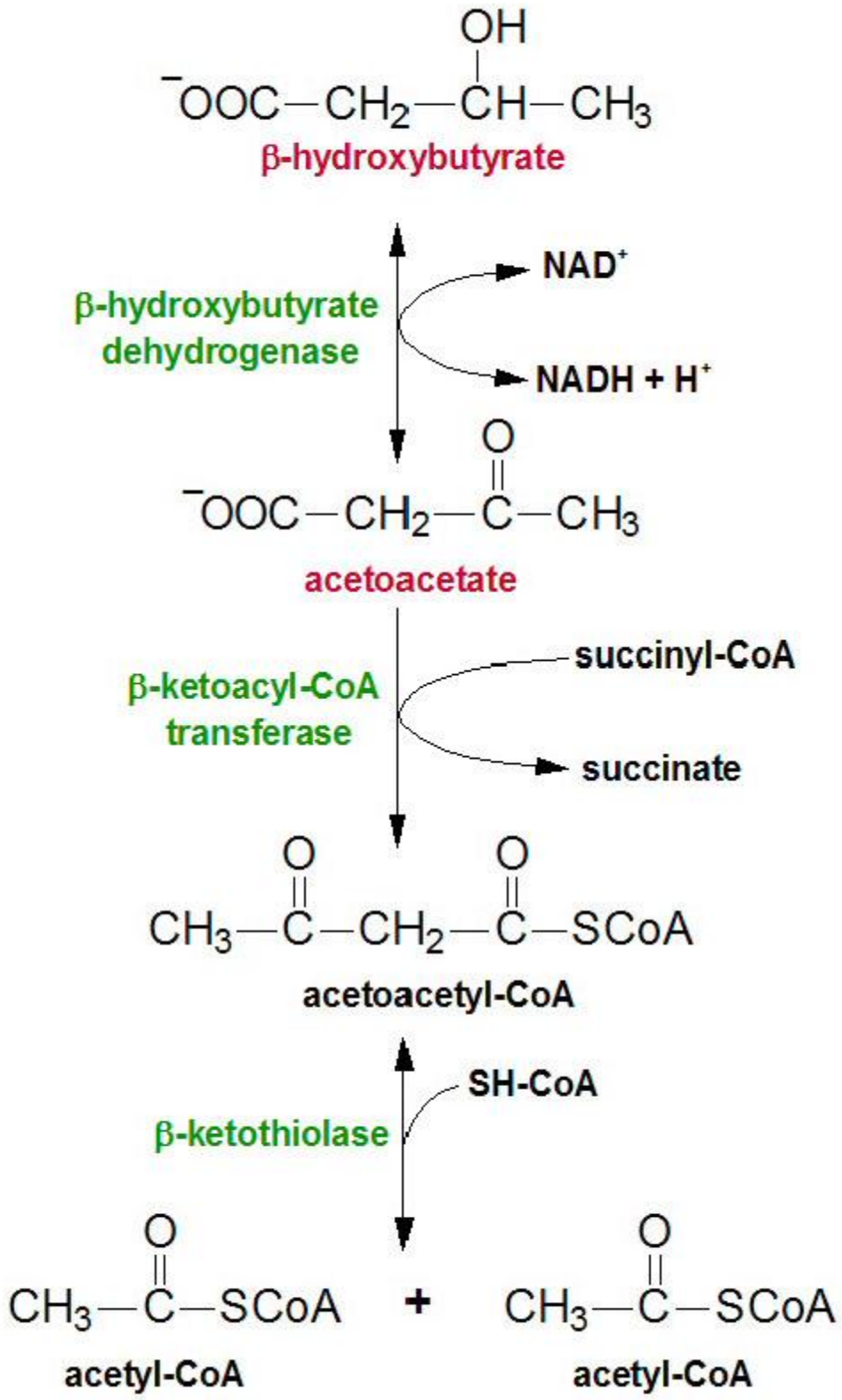


Synthesis of the Ketones

When the level of glycogen in the liver is high, the production of β-hydroxybutyrate increases. When carbohydrate utilization is low or deficient, the level of oxaloacetate will also be low, resulting in a reduced flux through the TCA cycle. This in turn leads to increased release of ketone bodies from the liver for use as fuel by other tissues. In early stages of starvation, when

the last remnants of fat are oxidized, heart and skeletal muscle will consume primarily ketone bodies to preserve glucose for use by the brain. Acetoacetate and β-hydroxybutyrate, in particular, also serve as major substrates for the biosynthesis of neonatal cerebral lipids.

Ketone bodies are utilized by extrahepatic tissues through the conversion of β-hydroxybutyrate to acetoacetate and of acetoacetate to acetoacetyl-CoA. The first step involves the reversal of the β-hydroxybutyrate dehydrogenase reaction, and the second involves the action (shown below) of acetoacetate: succinyl-CoA transferase, also called β-ketoacyl-CoA-transferase.



Utilization of the Ketones

The latter enzyme is present in all tissues except the liver. Importantly, its absence allows the liver to produce ketone bodies but not to utilize them. This ensures that extrahepatic tissues have access to ketone bodies as a fuel source during prolonged fasting and starvation.

Regulation of Ketogenesis

The fate of the products of fatty acid metabolism is determined by an individual's physiological status. Ketogenesis takes place primarily in the liver and may by affected by several factors:

1. Control in the release of free fatty acids from adipose tissue directly affects the level of ketogenesis in the liver. This is, of course, substrate-level regulation.

2. Once fats enter the liver, they have two distinct fates. They may be activated to acyl-CoAs and oxidized, or esterified to glycerol in the production of triacylglycerols. If the liver has sufficient supplies of glycerol-3-phosphate, most of the fats will be turned to the production of triacylglycerols.

3. The generation of acetyl-CoA by oxidation of fats can be completely oxidized in the TCA cycle. Therefore, if the demand for ATP is high the fate of acetyl-CoA is likely to be further oxidation to CO_2.

4. The level of fat oxidation is regulated hormonally through phosphorylation of ACC, which may activate it (in response to glucagon) or inhibit it (in the case of insulin).

Clinical Significance of Ketogenesis

The production of ketone bodies occurs at a relatively low rate during normal feeding and under conditions of normal physiological status. Normal physiological responses to carbohydrate shortages cause the liver to increase the production of ketone bodies from the acetyl-CoA generated from fatty acid oxidation. This allows the heart and skeletal muscles primarily to use ketone bodies for energy, thereby preserving the limited glucose for use by the brain.

The most significant disruption in the level of ketosis, leading to profound clinical manifestations, occurs in untreated insulin-dependent diabetes mellitus. This physiological state, diabetic ketoacidosis (**DKA**) results from a reduced supply of glucose (due to a significant decline in circulating insulin) and a concomitant increase in fatty acid oxidation (due to a concomitant increase in circulating glucagon). The increased production of acetyl-CoA leads to ketone body production that exceeds the ability of peripheral tissues to oxidize them. Ketone bodies are relatively strong acids (pK_a around 3.5), and their increase lowers the pH of the blood. This acidification of the blood is dangerous chiefly because it impairs the ability of hemoglobin to bind oxygen.